ChromStar 6

Manual

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for ChromStar 6.2 and higher versions

Contents

1. Introduction

The chromatography data system **ChromStar** together with an PC containing the ChromStar A/D converter board is capable of recording chromatographic data and operating chromatographic equipment such as solvent delivery systems, a UV detector or an autosampler. Some diode array detectors can be controlled and 2D data of these can be recorded and reprocessed.

The ChromStar software runs under the operating system of **Microsoft WINDOWS** and can therefore only be used in this environment (Windows 3.11,95,98, NT). Windows 98, 2nd edition will work, but is not recommended. The PCI A/D converter boards require as operating system **Windows NT, 2000, XP or Me**. The maximum user comfort offered by the windows technology is hereby achieved, such as the use of easily-accessed menus and dialogue boxes and, above all, the possibility of carrying out various jobs simultaneously, for instance recording of a chromatogram, while reintegrating an existing file and documenting the results. It is therefore advisable - although not essential - to be acquainted with WINDOWS when using the ChromStar software; this operating manual describes the steps necessary in sufficient detail. Normally, the most simple input method is described - a combination of keyboard and mouse entries.

This operating manual is not intended as an introduction into chromatography. Beginners are advised to consult the relevant literature.

The windows operating system exists in different languages. Depending on the language in use some menu procedures appear in other languages than described in the manual. ChromStar always uses easy understandable menu items in English. The on-line help pages are availanble in English or in German.

This manual describes the ChromStar version 6. Changes made in later versions will be recorded in supplements to the manual.

Chapter 2 deals with the **installation** of the ChromStar software and the accompanying A/D converter card.

Chapter 3 describes the **structure** of the ChromStar system, the various window techniques and the use of the keyboard.

Chapter 4 describes the **ChromStar software**, the menus and their submenus. The sections are named accordingly.

Chapter 5 shows how the various stages of **chromatography** are carried out with the help of the ChromStar system.

All **print-outs** that can be made with the ChromStar system - chromatograms, results lists, distributions plots etc. - are to be found in Chapter 6. Bold printed numbers in brackets in the text refer to these print-outs.

The following characters are not permissible when naming a new file: . , \ / : [ ] and the space bar. Avoid using national characters such as ä, ö, ü. All letters and numbers from a to z and 0 to 9 are permitted. The file extension (also called suffix) which is separated from the file name by a point is allocated automatically.

ChromStar exists in different versions:

ChromStar
ChromStar with DAD
ChromStar with GPC
ChromStar with DAD and GPC

DAD and GPC are additional options to the ChromStar full version.

The data systems **ChromStar *light*** and **ChromStar Integrator** are less comprehensive compared with ChromStar.

ChromStar *light* does not contain the *Transform* module.

ChromStar Integrator does not contain *Edit Files, Method, Autosampler Table* and *Edit Files, LC Procedure*, since ChromStar Integrator is not able to control any instruments. ChromStar Integrator does not contain *Edit Files, Preset*, the preset parameters can only be changed in the CHRST32.INI file (cp. p. 2-5). *Analysis, Select...* does not exist, since there is only one data acquisition channel. ChromStar Integrator does not contain the *Transform* module.

A number of print report templates are copied onto the computer during the installation, these can be used to get print-outs of the chromatograms. The **Report Editor** is an independent program to create new templates. A separate manual describes how to use the report editor program.

2. Installation

2.1 Description of the Hardware Components

For the ChromStar chromatography software a PC with a pentium processor (or better) with the following individual components is needed:

- RAM as needed by the operating system, a floppy disk drive, a CD drive and a hard disk
- a graphic card with a 800x600 resolution (VGA Graphic Card)
- a high resolution (800x600) graphics colour monitor
- a keyboard
- a mouse
- Microsoft WINDOWS operating system
  (version 3.11 is possible, recommended is Windows95/98 or NT, which need 16 Mbyte RAM)
- a Microsoft WINDOWS compatible printer

The ChromStar software package consists of:

- one or two A/D converter boards with plugs (depending on the configuration, one board for two channels or a one-channel-board, respectively).
- the ChromStar software CD
- a manual in English or German.

2.2 Installation of the A/D Converter board

Follow these instructions for installing the A/D converter board in your computer:

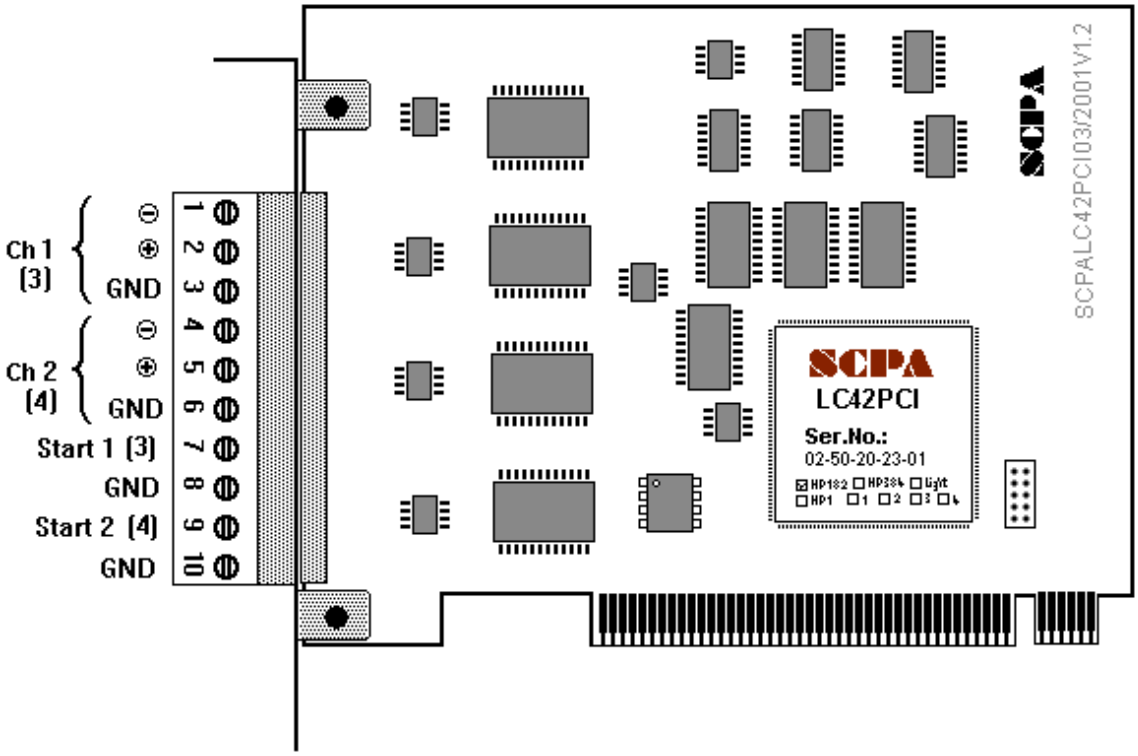
1. Switch off the computer and all attached devices (e.g. printers and monitors).
2. Remove the top of the unit. (If necessary refer to your computer user's manual.)
3. Remove the cover of a free slot.
4. Plug the converter board with its gold plated connector into the socket.

The second converter board for 4 channel recording requires its own slot.

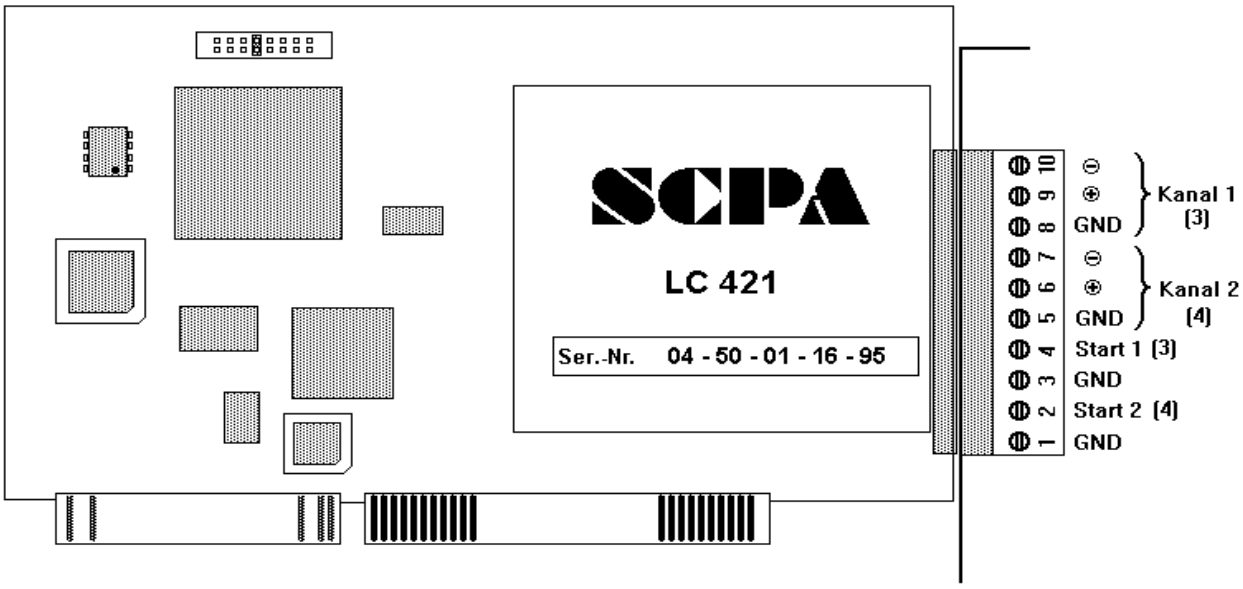
5. Screw the metal holder of the A/D converter board to the rear of the computer.
6. Close the top of the main unit. Reattach all cables you removed in step 2 and switch on the computer and its peripherals.

The PCI A/D converter boards require as operating system Windows NT, 2000, XP or Me. Windows 98, 2nd edition will work, but is not recommended.

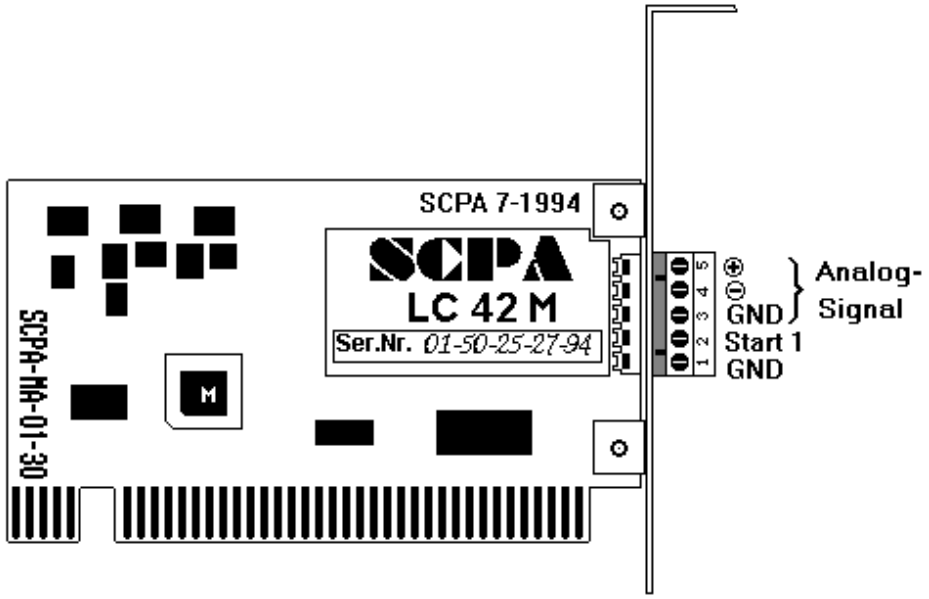
Pin assignment of the 2-channel PCI A/D Converter Board:



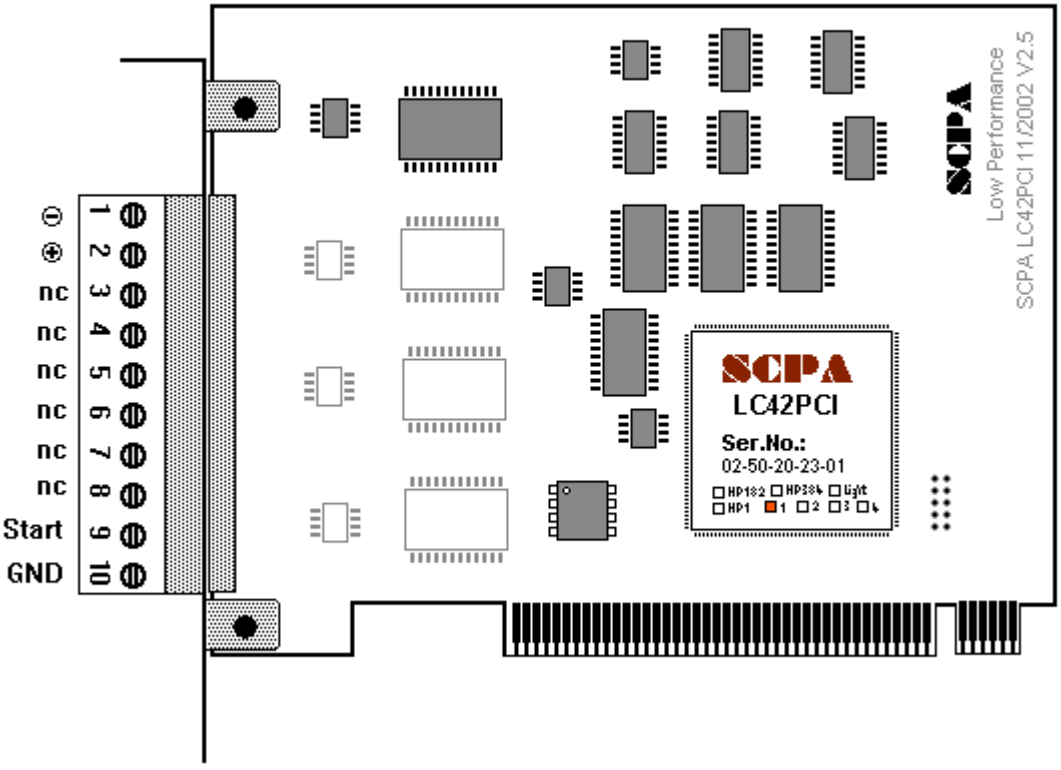
Pin assignment of the 2-channel-ISA-A/D-converter board:



Pin assignment of the 1-channel ISA A/D converter board of the ChromStar Integrator:



Pin assignment of the one-channel-PCI-A/D-converter board of the ChromStar Integrator:



nc = no connection

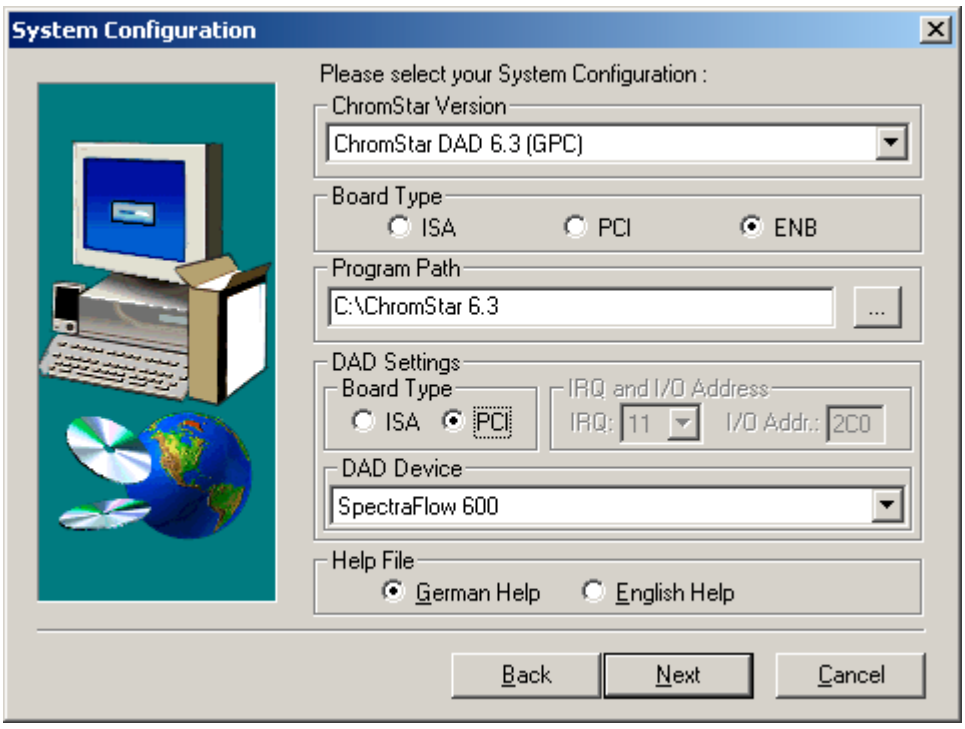
2.3 Installation of the ChromStar Software

Be sure that, before you install the ChromStar software, you first make a copy and store the original diskette or CD in a safe place.

Before installing the ChromStar software the WINDOWS operating system must have been installed. Follow the instructions for installation of this in the WINDOWS manual.

Insert the ChromStar CD in the CD drive and start the installation by double click on setup.exe. The **ChromStar-Setup** window appears. The ***Next***, ***Back*** or ***Cancel*** keys lead through the installation. The installation program is self-explanatory.

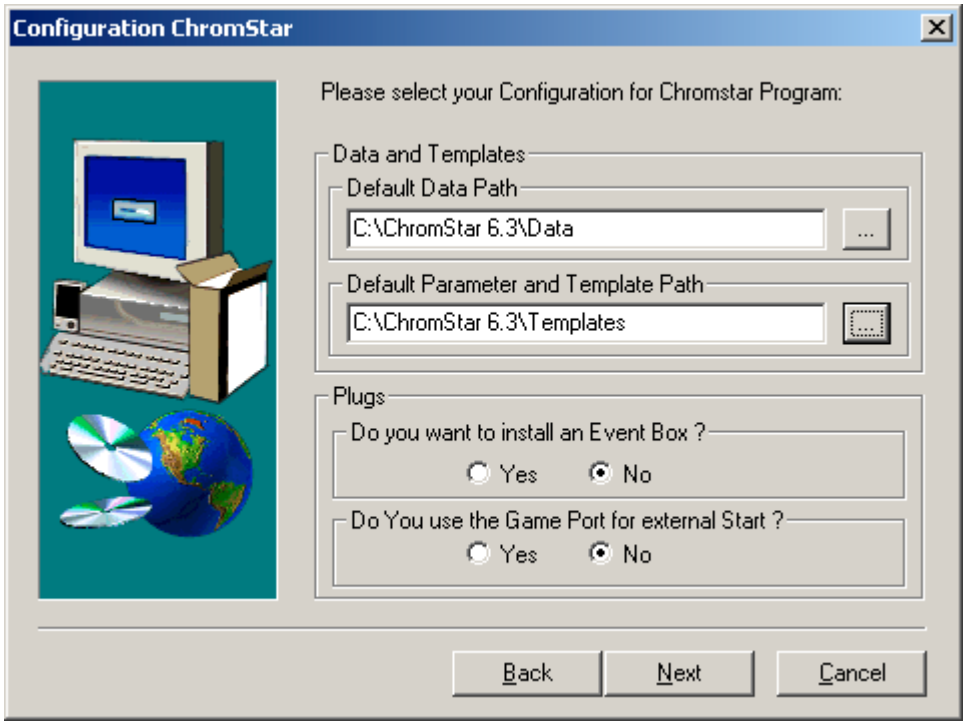
In the **GLP configuration** window you can choose wether you want GLP support or not. In the GLP-version the user's name and a password need to be entered. The password appears during entering as *****. The user whose name is entered at this moment will be the superuser. In every following ChromStar-start the user's name and his password are asked for, so that no unauthorised persons can use ChromStar.



In the **System Configuration** window you can choose the ChromStar version (downwards arrow on the right), the type of the A/D converter board (ISA, PCI or Ethernet-Box LC421 E), the program path, the type of the diode array detector (DAD) and the language of the help pages.

Depending on the ChromStar version chosen, the setup changes a little. In case of the DAD ChromStar detector details and parameters are to be entered. After choosing ChromStar Integrator the Printer Port Adapter can be installed. When the Ethernet-Box LC421 E is used the IP address must be entered in the next window.

In the **Configuration ChromStar** window the default data path and the default parameter and templates path are defined.



The use of the event box or of the game port is also defined here.

Using ChromStar with a DAD the instrument specific coefficients (for calculation of diode number to wavelength) must be entered in the **Calibration Coefficients** window. In the **Scan Presets** window some DAD specific preset values are entered.

Click Finish to complete the setup.

The ChromStar program files can either be stored in the WINDOWS directory or in a special directory (default C:\CHRSTAR) as desired. The CHRSTAR.FON, CHRSTAR.HLP, CHRGLP.DAT and CHRST32.INI files are copied automatically into the WINDOWS directory. The CHRSTAR32.EXE, COMSERV.EXE, REPORT32.EXE and ADINT.DLL files are stored in the ChromStar directory. The DDEML.DLL file must be in the Windows-SYSTEM-directory. The INIEDIT.CPL and COMMDLG.DLL files are also written into this directory.

After clicking in the program icon with the right mouse button and choosing submenu *Properties* the directory into which ChromStar has been stored is shown. When using the default directory the display states "C:\CHRSTAR\chrstar32.exe".

Some parameter settings for ChromStar are stored in the CHRST32.INI file. These are accessed and modified as follows:

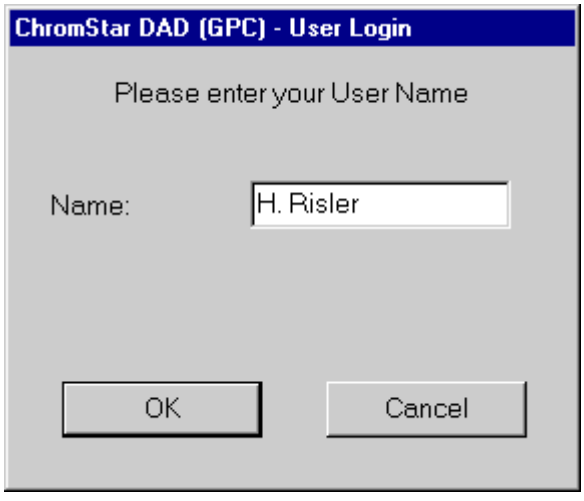
Call up the application *Notepad* in Accessories, select *File* and *Open* with your mouse and enter the file name CHRST32.INI. After confirming with OK the contents of CHRST32.INI appear in the window.

These are as follows:

[Directories]	
Data=c:\chrstar\data	Preset directory for ChromStar data-files
2DDATA=c:\chrstar\data	Preset directory for 2D data
Param= c:\chrstar\data	Preset directory for the selection of the data channels and for the report templates
Program= c:\chrstar	Preset directory for the ChromStar program
[Printing]	
logo=no	No logo appears on print-outs in Transform.
GLPReport=yes	With GLPReport=yes an extended protocol is created during printing in the GLP version, it can be switched off with GLPReport=no.
Pen=x	x=0, 1, 2 .... pen width in some print-outs.
[Preset] ...	Contents of the preset table
[Configuration]	
eventbox=no	No if an event box is not to be used. Alternatively change to yes.
COM1=LC:1;AS:3	This part shows the configuration of LC devices. In this example the instruments are connected to the RS232 interface COM1 (=LC1 in the selection). The pump (LC) has the device number 1, the autosampler (AS) device number 3. The LC devices are connected by a nine pole flat cable.
DISABLEAUTOSAMPCOM1=no	An autosampler is in use
AllowMulti-SelectForMulti=1	multiple file selection using Shift or Strg
[SCANPRESET] ...	Preset parameters of the diode array detector
[PARAM22COM1] ...	Instrument parameters for a controllable LC pump
[Document table] ...	Entries in the documentation page of the method file (cp. 4.1.1.4.).

[COLORS] ...	Colors in ChromStar, can be changed in ***Edit Files, Chrom.Files, Colors***.
[Calibration] p0 ... p4	Coefficients of the calibration function for calculating the wavelength using the diode number of the diode array (individual diode array detector parameters, these are supplied with each detector)
[mms] range=normal wide ultra	Wavelength range of the diode array detector 200 – 400 nm 200 – 640 nm 200 – 1020 nm
[Board] Channel 1&2=1 Channel 3&4=7	A/D-Board in use. Example: LC421 is installed No A/D convertert board is installed. This entry is necessary for 1&2 when only a DAD is used.
[WindowPositions]	On leaving ChromStar the window positions are stored.
[Channeldef1]	Representation of a detector signal (from channel 1 to 4)
[Colcoeff] Calculation=enabled Factor=2	 Calculation of column coefficients Factor for the calculation of the resolution

After modification the entries can now be stored by clicking *Files* and *Save*. Exit the application by clicking into *Close*. ChromStar must be restarted before some of the modifications can take effect.



In the ChromStar start dialog of the GLP version the user's name and password has to be entered in agreement with those entered during the installation. In the none-GLP version only the user's name or nothing can be entered.

The appearance of this window can be switched off if always the same user works with ChromStar. This is done as follows: click on the program icon with the right mouse key and in the menu *Properties.* Extend the Command Line (C:\...\chrstar32.exe) with the user's name.

This name appears later with every recorded chromatogram as author‘s name.

A double click on the ChromStar icon and answering of the ChromStar start dialog causes ChromStar to start with its title page.

3. Description of the ChromStar Window

3.1 ChromStar Structure

The ChromStar system consists of 7 main applications with the following functions:

With **Edit Files** the parameter-files necessary for recording and reprocessing of chromatograms are edited and stored.

Analysis controls the recording of chromatographic data by e.g. a UV detector (**Chromatogram**) or by a diode array detector (**DAD**).

Reprocess allows chromatographic data to be reprocessed and re-presented (**Integration**), allows the quantitative evaluation of chromatograms, the reprocessing of groups of chromatograms, the comparison of series of chromatograms and data-conversion (**Calculations**). DAD data (**2D**) and spectra (**Spectrum**) can be represented and reprocessed.

Transform allows you to add or subtract two chromatograms, to calculate derivatives, to correct baselines and to deconvolute peaks.

GPC accesses all files necessary for evaluating chromatograms recorded by means of gel permeation chromatography. Molecular weight and the specific GPC calculations on GPC chromatograms can be carried out here.

Under **GLP Options** in the GLP-version new users and new passwords can be registered.

The menu procedure ***Extras, Options*** opens a window where optional settings for some ChromStar applications can be made. These settings have a corresponding entry in the CHRST32.INI file. The meaning of the settings is given in the appropriate places in this manual. The menu procedure ***Extras, Options*** does not appear when the CHRST32O.DLL is not present or has been deleted.

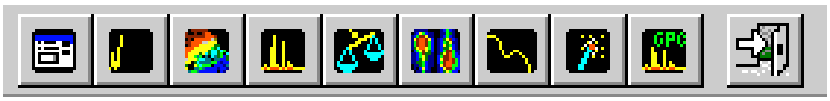
3.2 Operating Elements

With a double click on the program icon the Start dialog is activated. After entering the correct information the ChromStar-Window (fig. page 2-7) appears on the screen.

The **title bar** contains the name of the application. If the window is active it can be moved around the screen by clicking in the title bar with the mouse and holding the button down whilst moving the mouse. The full picture cannot be moved however. A light blue title bar marks an inactive application. Clicking any position within an inactive application activates it whereupon the title bar is shown in dark blue.

The **menu bar** contains the current submenus available. After a submenu has been selected its menu bar appears above the relevant box. The procedure option ***Window*** and its submenu ***New*** allow you to open further sub-windows. The contents of the menu box change according to the window activated. A series of windows can be displayed side by side or in an overlay pattern by the ***Window*** submenus ***Tile*** or ***Cascade***.

The **toolbar** contains buttons to facilitate the use of the most important functions of ChromStar. Each button corresponds to a menu or submenu procedure. The buttons for the main applications of ChromStar are situated to the right. The exit button closes the active application and leads to the next higher application.



The meaning of the buttons is as follows:

Edit Files
Analysis - Chromatogram
Analysis - DAD
Reprocess - Integration
Reprocess - Calculations
Reprocess - 2D
Reprocess - Spectrum
Transform
GPC

The meaning appears in the **information line** at the bottom of the ChromStar window as soon as the mouse cursor is moved over a button.

The **frame** of a window has various functions too. Moving the mouse over the frame brings a slanting double arrow at the upper and lower rim of the frame and a horizontal double arrow at its right and left side. A window can now be enlarged or reduced by depressing the left button of the mouse, moving it in the required direction and then releasing it. When the window is reduced other windows previously opened become visible.

In the top left hand corner of the screen is the **Control Menu Box**. Placing the mouse arrow in this field and clicking the left mouse button calls up a list of operations which can be carried out e.g. clicking *Minimize* causes the window to become an icon, clicking Maximize expands the window over the entire screen, clicking *Close* shuts down the window. A sub-window can also be closed down by a double click on the Control-Menu box. The Control-Menu Box functions can also be executed with a combination of keys. If none of the functions of this box are accessed, it can be exited by striking ESC or by clicking any part of the screen outside the Control Menu box.

A sub-window can be transformed to an icon by clicking the **Minimise Box** (the second box from the right). The icons of the sub-windows are to be found at the bottom of the ChromStar window.

A double click onto an icon restores its window to the screen.

A single click recalls the Control Menu box.

The **Maximise Box** at the top right hand corner of the screen allows you to expand an application up to the full size of the screen. The title bar then contains the title of the application besides the ChromStar symbol. The switches are now in the title bar and in the menu bar appears a switch with an up/down arrow. Pressing this switch restores the application to its previous scale. It also allows you to switch between a large and a reduced-size window.

Clicking and holding the title bar allows you to move a window so that a part of it gets outside of the ChromStar window and becomes invisible. At that moment at the right hand and lower sides of the ChromStar window **scroll bars** appear which allow operations outside the window area to be carried out.

Individual windows can be closed by clicking ***Exit*** or use the Exit button.

Applications and submenus displayed in light colours are not accessible at the present stage of operations.

After clicking the Minimise Box next to the ChromStar title bar, ChromStar appears at the bottom of the screen in the task bar. All opened sub-windows are included in it. A click on the task icon restores the previous status.

3.3 Menupoint GLP Options

GLP Options is active in the GLP-Version and controls the accessibility. This menu procedure only appears when ChromStar is installed in the GLP mode. It allows new users and passwords to be introduced.

User Administration

In the ***User Administration*** window the users' access rights are determined. In the window to the right the activities can be chosen by clicking into the appropriate item or sub-item. Using the ***Apply*** key the changes become active.

With the ***New User*** key a new user with limited accessibility can be entered. After entry of name and password a user level is chosen: Administrator, Lab-Manager or User. The Super User had installed ChromStar previously and has no further rights. With the ***Delete*** key a user can be removed from the users list.

Change Password

Change Password allows the user, who already opened ChromStar with his name and password, to change this password.

Change User

With *Change User* another user, who was registered before with name and password under *User Administration* and *New User*, can take over the ChromStar control.

Forbidden Passwords

Forbidden passwords can be determined here.

The points ***User Administration*** and ***Forbidden Passwords*** are only accessible for the Super User and the Administrator. After installing ChromStar in the GLP mode the Lab-Manager has all rights and the user almost none. The access rights can be changed in the ***User Administration*** window as required. In the GLP mode the program is frozen after a certain time and can only be reanimated by using the ***Log on*** key and entering the password. Immediate freezing of the program can be achieved by pressing ***Window*** and its sub-menu ***Log out***. The interval, after which the program is automatically frozen, can be defined in the CHRST32.INI file, as well as the time after which the password is no longer valid. Entries in CHRST32.INI in the section [GLP]: PwdTimeOut=xx defines the time in days after which the password expires. TimeOut=yy defines the time in minutes after which the program is frozen.

3.4 Menupoint Window

The menupoint ***Window*** allows you to choose how to display a number of opened applications on your screen.

Meaning of the submenu:

New

New enables you to open another ChromStar application. Selection of the desired application and clicking OK causes the menu list to change accordingly.

Tile

The *Tile* command arranges the open windows in smaller sizes to fit next to each other on the screen.

Cascade

Cascade causes the open windows to overlap so that each title bar is visible.

Arrange Icons

Arrange Icons arranges the icons of opened applications at the lower edge of the ChromStar-window.

Close All

Close All closes all applications within ChromStar.

Log out

Clicking *Log out* causes the application to be blocked. Keyboard entries are no more effective. In the menu list only the menu point ***Log on*** appears, which can be used to reactivate the application. In the GLP-Version entry of the password again is necessary.

A list of all opened applications is shown in the bottom of the submenu of ***Window.*** The active application is marked. An application can be activated by clicking into this list.

3.5 Help pages

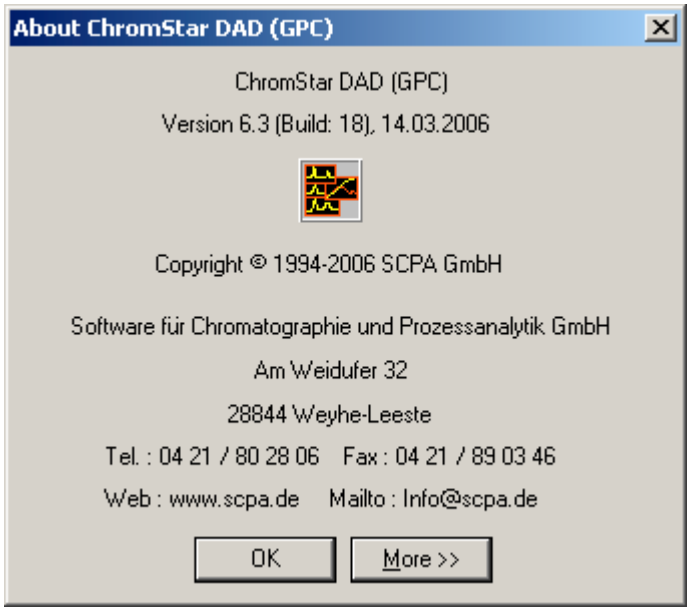
With ***Help*** the help pages can always be accessed from every ChromStar application. The language of the help pages is defined during installation of the ChromStar software.

With ***Help Context*** the help page for the currently used application appears.

With ***Help Contents*** the first page with explanations about the main menu points is opened.

With ***About ChromStar*** a dialog box is displayed which shows the information about the version number.

The *More* key addionally shows version number and date of origin of the installed ChromStar files.



3.6 Function Keys

The Function keys F1 - F12 can be used to accelerate handling when recording data in the **Analysis** application. The application must be active. They have the following functions:

F1	START	starts a chromatographic run in a method.	
F2	STOP	stops the chromatogram which is evaluated and printed up to the time of stopping it.	
F3	ABORT	interrupts the chromatogram without storing and evaluating the data recorded.	
F4	CONT	stops data-acquisition and starts integration and printing. An LC-Procedure is further finished off.	
F11	SCRN SCALE	allows you to change the screen scale of the detector signal in an entry box.	
F12	NORM	normalises the screen display of the detector signal so that the lowest value is displayed.	

The following function keys control an HPLC pump:

F5	SOLV A	Solvent A is pumped.	
F6	SOLV B	Solvent B is pumped.	
F7	SOLV C	Solvent C is pumped.	
F8	SOLV D	Solvent D is pumped.	
F9	STOP PUMP	The pump is stopped so that no further solvent is pumped.	
F10	FLOW RATE	The flow rate can be altered in an entry box.	

If you prefer to use the mouse instead of the function keys, you can carry out all operations described here by clicking the various commands in the menus and submenus.

3.7 File Extensions

When recording and evaluating data with ChromStar various types of files are created to which automatically a file type description is added as an extension, separated from the file name by a dot. These file extensions and the files they belong to are listed below. A detailed description of the file contents will be found in Chapter 4 where the ChromStar menus are described.

Method	MET	chromatographic method
Data Handling	INT	integration parameters
Calculation	CAL	quantitative evaluation, calibration
LC-Procedure	PRO	pump control
Preset	INI	default parameters
Scan Parameters	DAM	parameters for the use of a diode array detector
Selection	RSE	selection of data acquisition channels
Error	ERR	error messages during data acquisition
Slices	SLI	chromatogram (raw data)
Report	RPT	results of the last calculation is **always** created together with the .SLI-File.
2D-Data	DAD	2-dimensional set of data recorded by means of a diode array detector
Spectrum	DAS	UV spectrum, extracted from the 2D data set
Report Format	RPF	report print templates
	RPD	
	RPC	
	RPL	
	RPG	(GPC)
	RMG	(GPC)
	RPA	(for DAD data)
	RPS	(for spectra)
GPC- Data Handling	GIN	parameters of the molar weight evaluation
GPC-Calibration	GCA	calibration data for molar weight evaluation
GPC-Batch	GBA	evaluation of series of GPC chromatograms

4. Description of the ChromStar Software

4.1 Edit Files - Creating and Editing of Files

The application window **Edit Files** permits you to create new or change existing files necessary for recording and evaluating a chromatogram or a 2D data set.

The files required for carrying out the analysis, integration and quantitative calculation have the following function:

Method. The Method-File contains the necessary entries for the execution of an analysis. Registered here are the name of the chromatogram, the processing (HPLC/GC or GPC), the type of print out and the names of the LC-Procedure and Data-Handling files. When an autosampler is used the sequence and vial handling used are specified here. Further on entries can be made here, for each injection, about the type and quantity of the sample. It is also possible to write notes concerning the analysis in a document table.

Data Handling. In the Data-Handling-File parameters are defined for recording of the raw data and for the integration. The page with data acquisition parameters contains for example the run time of a chromatogram, the slice width (time between the data-points) and the data storage parameters. For quantitative calculations a Calculation-File can be specified here. The integration parameters such as *skim ratio*, *noise* and *threshold* can be programmed chronologically, i.e. they can be modified during the course of a run.

Calculation. The Calculation-File contains the necessary parameters and entries for calibration and quantitative evaluation. On the first page of this file the calculation method (percent, normalisation, internal or external standard) is specified, the type of time window (absolute or relative retention time), the calculation basis (peak area or height) and the number of calibration samples. The second page contains the peak table with the retention times, response factors and peak names. Also the amount of the individual components of calibration samples is entered here. On the third page regression coefficients for a multi level calibration are stored.

LC Procedure. In the LC procedure file the parameters for the control of HPLC equipment via interface (as solvent composition, flow rate, oven temperature, detector wavelength etc.) are defined in a time table. Each parameter can individually be adjusted at different times for instance to generate solvent gradients, to change the flow rate or to record a UV spectrum.

Scan Parameter. In the scan parameter file the name of the 2D data set, parameters of the diode array detector as wavelength range and resolution and the run time of the data acquisition are defined.

Preset. In the preset file the default values of certain parameters like flow rate or wavelength are specified. These are always used if they have not been defined in one of the files mentioned above. The specified parameter values can be changed and stored (in the CHRST32.INI file), they are automatically activated after the next system start.

The menu bar of the Edit-Files-window contains the points

Chrom.Files Options Exit Edit Files Window Help

Chrom.Files with its submenu

Method Data-Handling Calculation Scan-Parameter LC-Procedure Preset Colors Show Connect...

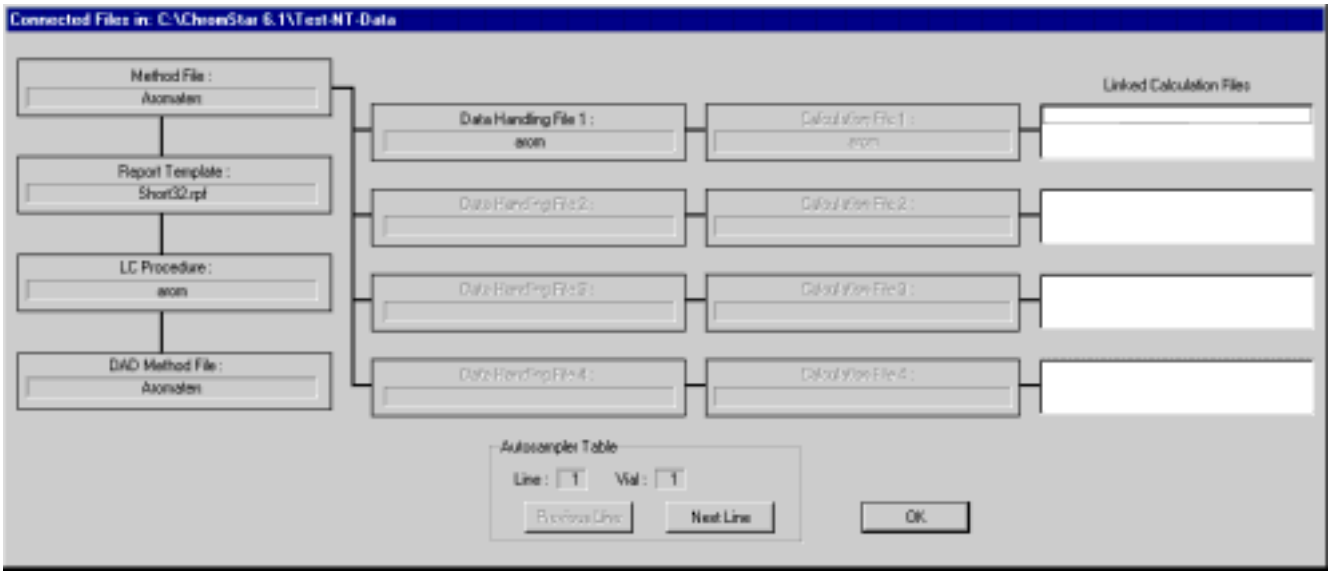
allows entry to the individual file types. These are described further in detail in their own chapters.

Buttons in the toolbar allow quick access to the files.

Colors opens the **Colors** window, in which for the various representations on the screen (**Screen Colors**) or for the print-out (**Print Colors**) the colors can be individually defined by the user. After clicking a function on the left a color can be selected either under **Basic Colors** or in the mixing area. The color selected in the mixing area is shown in the **Color/Solid** box at the bottom to the left. Some functions can only be represented in a **Solid** color. The selected color is immediately shown in the box on the right to the function. Using **OK** all selected colors are saved in the CHRST32.INI file and are used in ChromStar for the various representations. The **Set Default** key restores the original ChromStar colors. Using **Cancel** the window is closed without changing colors.

Show Connect shows all further used files of a selected Method-File like Data-Handling, LC-Procedure and Calculation-Files (except for Mode = GPC).

When an autosampler is used all files belonging to the individual vials in the Autosampler Table can be viewed with the switches *Next Vial* and *Previous Vial*.



Options contains the submenu

Print Copy Search for Sample Id

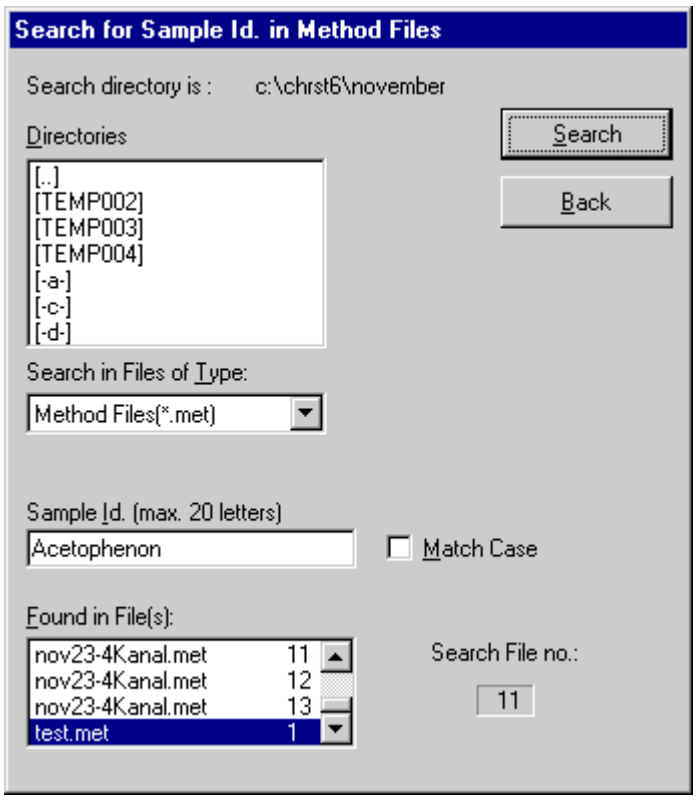
With ***Print*** or the  button one of the file types can be printed (print-outs of all file types protocols **1 - 5**), with ***Copy*** one of the file types can be copied into the Clipboard.

A submenu with a list of the different file types appears after clicking ***Print*** or ***Copy***. The desired file type is selected here.

After this a dialog box appears from which a file can be selected.

After clicking it the file name appears on a black background and in the box above. Clicking OK starts printing, clicking *Cancel* breaks off this action.

Search for Sample Id. can be used to find a *Sample Identifier* (cp. Method File - Sample Table) either in a method file or in a chromatogram.



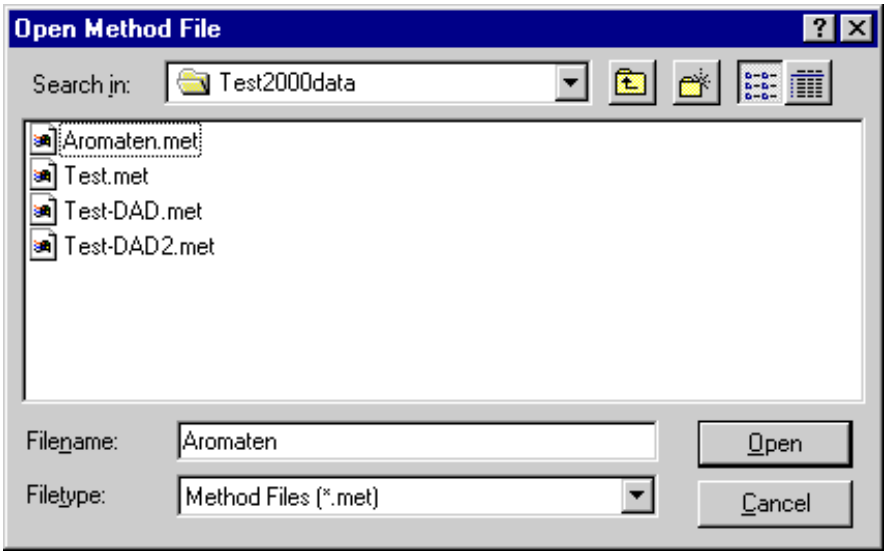
The Menu points ***Window*** and ***Help*** are already described in the chapters 3.4 and 3.5.

The **Edit Files** application window can be reduced to an icon by clicking its minimize button (third field at top right corner).

You quit **Edit Files** with ***Exit Edit Files***.

4.1.1 Edit Files - Method

Clicking ***Chrom.Files*** and ***Method*** or using the button met accesses the dialog box **Open Method File** for entering the name of an existing or of a new file.



Out of the list of all existing files a file is chosen or a new name is entered in the entry field after *File Name.* The box on top after *Search in* shows the chosen directory. The downwards arrow shows the list of all directories. The upwards arrow leads to the next higher directory.

If a new file is to be created its name (up to 23 characters) is entered in the entry box against *File Name*. It is not necessary to define the file type.

An existing file can be selected from the list by clicking it, whereupon its name appears in the entry box and on a black background in the list.

You can overwrite a file name entered in the entry box by moving the cursor with the left mouse button depressed over the box. The name appears on a black background and can now be overwritten.

The file selection is confirmed with *Open* or RET.

Now the selected Method file (Run Table cp. fig. on page 4.1-7). is displayed.

Cancel closes the file select box without opening a file.

The menu bar contains the menu points

File Window Help

The individual pages of the Method-File accessible via index cards are described in Sections 4.1.1.1 to 4.1.1.4.

The menu point ***File*** corresponds to the button  and appears for all files which can be edited with ***Edit Files***.

The menu points ***Window*** and ***Help*** appear on all ChromStar applications (detailed description see chapter 3.4 and 3.5).

File contains the submenu

New Open... Save Save as... Print Printer setup...

Copy File Information... Close

and allows file operations.

With ***New*** the first page of a Method-File with the name "Untitled" appears. This can now be edited and with ***Save As...*** be stored under a new name.

With ***Open*** a file can be opened to make changes or to create a new file. To secure the file after changing the question appears "Save...file?", after answering yes the selection field "Save ... File As" appears where the desired filename can be selected from the file list.

Save allows a changed or new file to be stored.

Save as... allows a changed file to be stored under a new name.

Clicking ***Print*** or pressing the button  prints-out the file (cp. printer protocols **1** - **5**).

Printer Setup opens a dialog box in which the printer setup can be changed.

Copy copies the file into the clipboard.

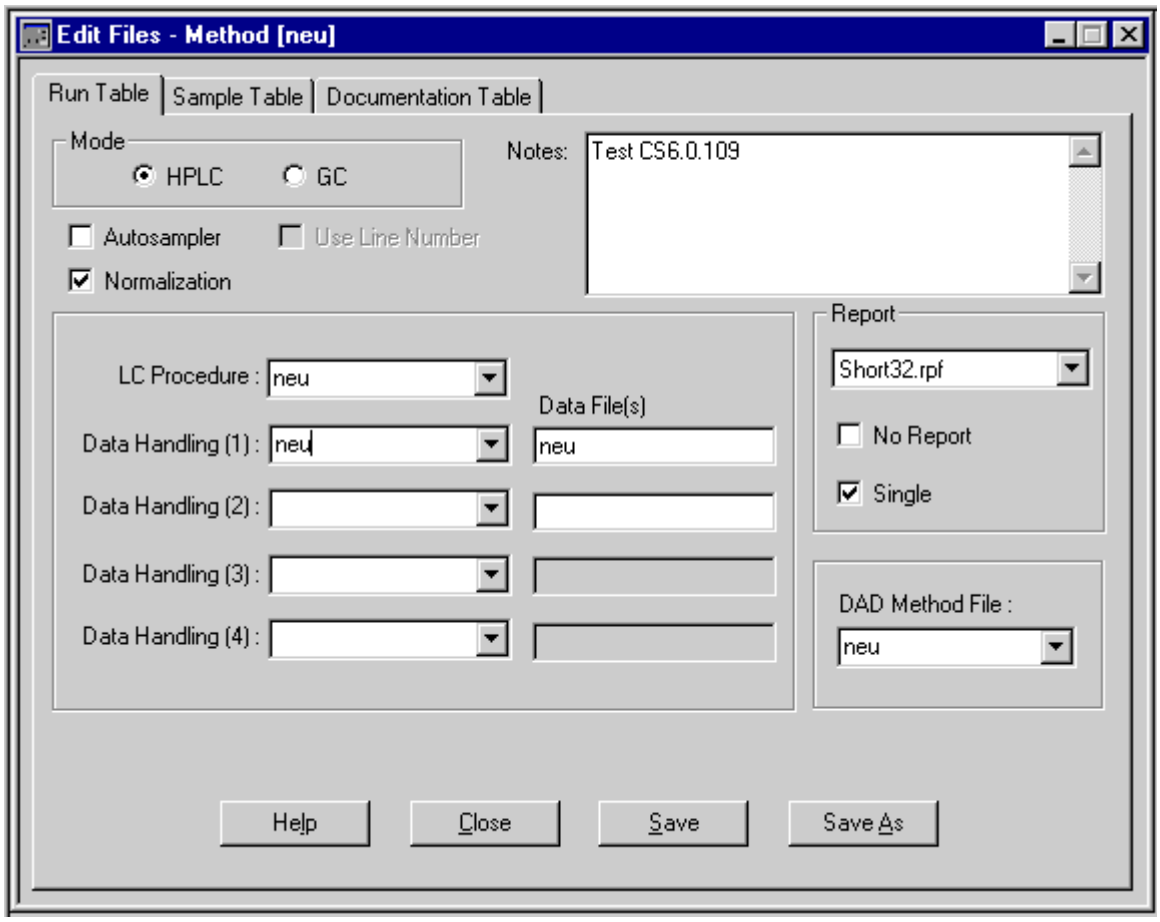
With ***File Information*** the author name, date of origin and date of the last changes of a file can be viewed.

Close Method allows you to leave a processed file. If changes have been made the question is asked whether the file has to be saved or not.

The different pages of the Method file – **Run Table**, **Autosampler Table**, **Sample Table** und **Documentation Table** – are accessed by clicking with the mouse into the cards. Access via key entry is possible as well. CTRL+TAB moves to the next card to the right, CTRL+Shift+TAB moves to the next card to the left. The different pages of the data handling file and of the calculation file are accessed in the same way.

Clicking the Minimise Box in **Edit Files** - Method iconises the application; the icon EF appears at the bottom of the ChromStar window and the file can be further viewed and processed later. It is not possible to open more than one **Edit Files** window so only one file at a time may be edited.

4.1.1.1 Edit Files - Method - Run-Table



The parameters on this table have the following meaning:

Mode (HPLC/GC/GPC) determines how the chromatogram has to be evaluated. HPLC and GC mode lead to a calculation of the peak areas, heights and retention times. The GPC mode performs a molecular weight calculation and requires an other data handling file (Section 4.9.1.1). A chromatogram for calibrating the GPC column must be recorded in HPLC-Mode. Once you have clicked in to GC the parameter *LC Procedure* can no longer be accessed as these are used to control an HPLC device. In addition, the Documentation Table of the method file changes and permits the entry of specific GC notes. The report forms under Report change according to whether you have clicked HPLC/GC or GPC. The mode required is chosen by clicking the appropriate button with the mouse.

Notes. Several lines for notes concerning the analysis e.g. sample preparation, column, eluent etc. can be entered here.

Autosampler. This parameter defines whether a with ChromStar controllable autosampler is used or not (for autosampler control see also section 5.1.4). If this box is selected the parameters *LC Procedure* and *Data Handling (1)* to *Data Handling (4)* appear in a light grey print which means that they cannot be accessed. The names of the data handling and LC procedure files to be used for each individual vial are now entered in the Autosampler Table (cp. next section).

Autosampler must be clicked in a calibration run when the calculation is to be carried out immediately after recording the chromatograms, even when no autosampler is in use. The standard is marked in the autosampler table under type as S (cp. P. 4.1-11). (For more details cp. p. 5-10 and 5-27ff.)

Use line number. When this item is marked the line number of the autosampler table is used to build the name of the chromatogram (Data Files, s.below.), namely the 5th character of the file name corresponds to the line number (1 - 9, A = line 10, B = line 11 etc. up to Z). The AS table may only contain max. 35 lines, the 6th and 7th character is the vial number, the AS may only contain up to 99 vials. The 8th character is the injection number (max. hexadecimal 15=F). The item *Use Line number* should be marked, if a vial is entered in different lines and the chromatograms are used later on for a common calculation, e.g. multi-level calibration.

Normalization. This parameter defines whether the print-out of the chromatogram should be normalised to the highest peak or not. No normalization means that the scale of the y-axis is determined by the *Screen Scale* factor (section 4.1.2.1). In this case the chromatogram is printed exactly as it appears on the screen.

Clicking the check box results in switching between Normalisation and No Normalisation.

Report (No report or selection of a report template. This entry defines how the chromatogram is to be printed. A report print template is chosen from the list of standard templates or templates created by the user (Protocols **6** - **8**, section 6.2, for GPC cp. section 6.8). Entering *No Report* means that no print-out is produced, the chromatogram, however, is saved.

In a multi-channel data acquisition each chromatogram is printed out on one page if *single* is clicked. In the other case a print report template can be chosen for the representation of several chromatograms (.RPD or .RPC) (**14**, **15**, **16**).

LC Procedure. This field allows entry of the LC procedure file which is used for the analytical part of a method and which controls an LC pump. If no LC pump is being used it is not necessary to enter a name here. The name of the LC procedure file of up to 8 characters automatically receives the name of the method file by default. You can, however, use any other LC procedure file or enter an other name by over striking the default name with the cursor while keeping the left mouse button depressed. A black background appears and now the new name can be entered.

Data Handling (1 to 4). Here the names (up to 8 characters) of the data handling files are specified. The default name for channel 1 is that of the method file. When two or more channels are being used to record data, the data handling file of the second and further channels must receive different names to avoid overwriting the chromatograms of the other channels. When using more than one channel the entries must be made one after another, i.e. no data handling box may be passed over with the TAB key. The channel numbers of the A/D boards do not necessarily have to correspond to the numbers 1 to 4 here. The Data-Handling-Files must have been specified before the analysis is started.

Data File(s). Behind the Data-Handling-file for each channel the name of the chromatogram (up to 19 characters, different names for each channel) is entered. Each injection receives automatically an increasing number starting from 0001. If an

autosampler is used the digits 5 until 7 are used to store the vial number. Digit 8 is used to count the number of injections from the same vial (expressed in hexadecimal numbers, maximum 15 = F).

Chromatograms (.SLI) and at the same time Report-Files (.RPT) are saved under these names.

DAD Method File. When using a diode array detector the name of the scan parameter file to be used is specified here. This file contains the parameters for data acquisition by the diode array detector.

Entry of parameters is done again after clicking the selected fields with the mouse or by using the TAB key, with the TAB key you step to each next field. Shift + TAB allows you to step backwards.

Wherever in a ChromStar file a file name is entered as parameter this can be done in two ways: Either the name is entered in the entry box or the downwards arrow to the right of the box is clicked and the file name is chosen out of the list of the existing files.

The LC-procedure, Data-Handling-Files and Scan Parameter file specified in the Method-File must first be created in the same directory as the Method-File before the analysis specified in the Method-File can be started.

The **Close**, **Save**, **Save as** and **Help** key correspond to the menu procedures ***Close***, ***Save***, ***Save as*** and ***Help Index*** and allow quick file editing.These keys are present on all pages of the ChromStar files and have the same function as described here.

After creating its four pages the Method-File first needs to be saved before executing an analysis. Pay attention that only entries on the first page are necessary to carry out an analysis, saving the default entries is enough.

The file can be saved with ***Save*** or ***Save as*** commands under the menu point ***File*** or by using the appropriate screen keys.

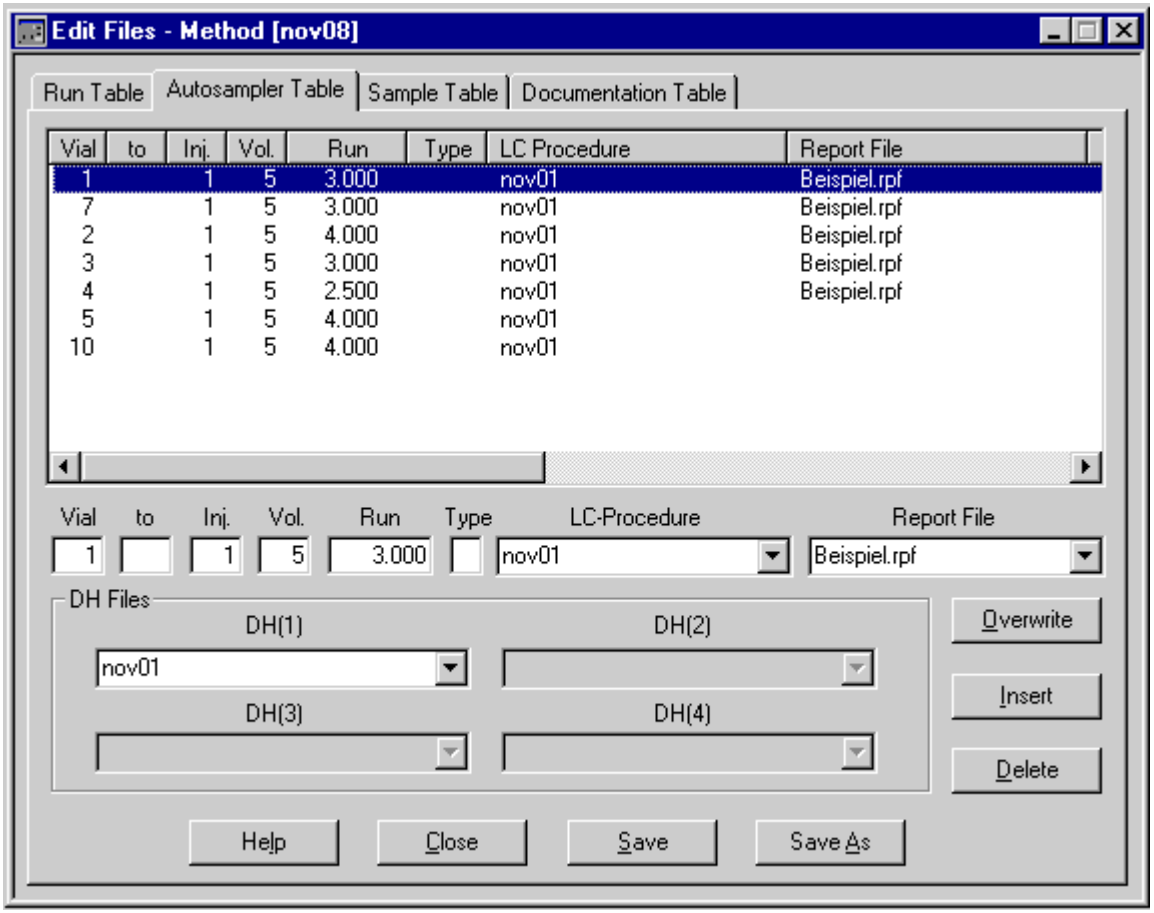
After carrying out modifications and clicking the submenu commands ***New***, ***Open***, or ***Close Method***, a message appears asking whether the changes have to be saved or not.

Using ***File*** and ***Open*** or the button  another file can be selected and be opened.

Using ***File*** and ***Close*** or the button  the file is closed.

4.1.1.2 Edit Files - Method - Autosampler-Table

The order in which the samples are to be processed is specified in this table. The table can only be accessed if the parameter *Autosampler* is marked in the Run Table.



The parameters of this table have the following function:

Vial. The number of the vial to be processed, entry starting from 1 up to a maximum value which depends on the available autosampler. With "W" a washing procedure is executed without an injection from a vial (also depending on the type of autosampler). The duration of the washing procedure is entered in column 5 (Run) and the solvent composition is specified in the LC-Procedure-File mentioned in the LC-PROC column.

to. Depending on the available autosampler a vial number can also be entered here (2 or higher). In this way a continuous series of vials is processed, starting from the vial number entered in the first column until the vial number entered here.

Inj. The number of injections (1 – 15, depending on kind of autosampler in use) to be made from a vial. If a series of vials have been entered in the first two columns the number of injections specified here are first carried out from the same vial before the next vial is processed.

Vol. In this column the sample volume to be injected is entered in µl. The smallest sample volume which can be entered is 1 µl, the largest 50 or 250 µl depending on the syringe type installed in the autosampler and specified in the Preset File (Section 4.1.5). The injection volume is stored in the Slice File Header and can be printed out (object Inj. Volume, cp. Report Editor Manual).

Run. Run time (in minutes, three decimals) of a chromatogram, after this time data-acquisition is stopped.

Type. If one or more samples are used as a calibration standard they must be marked in this column with "S".

LC-Procedure. Entered here is the name of the LC-Procedure-File with which the samples of this line are to be analysed.

DH(1) bis **DH(4).** This name specifies the data handling files to be used on channels 1 to 4 for the samples of this line.

Entries are made in the entry bar beneath the table. The next field is accessed with TAB or by clicking with the mouse. Shift+TAB moves the cursor back to the previous box. File names can be accessed using the downwards arrow. Clicking *Insert* inserts the entry line to the table. If a line already present in the table is clicked, it appears on a black background and in the entry line below. It can now be edited and re-entered in the table by clicking *Overwrite*. By clicking *Insert* the line will be inserted after the old one. A marked line can be deleted with *Delete*.

If the autosampler table has more than 10 lines a scroll bar appears to the right with which you can move through the table.

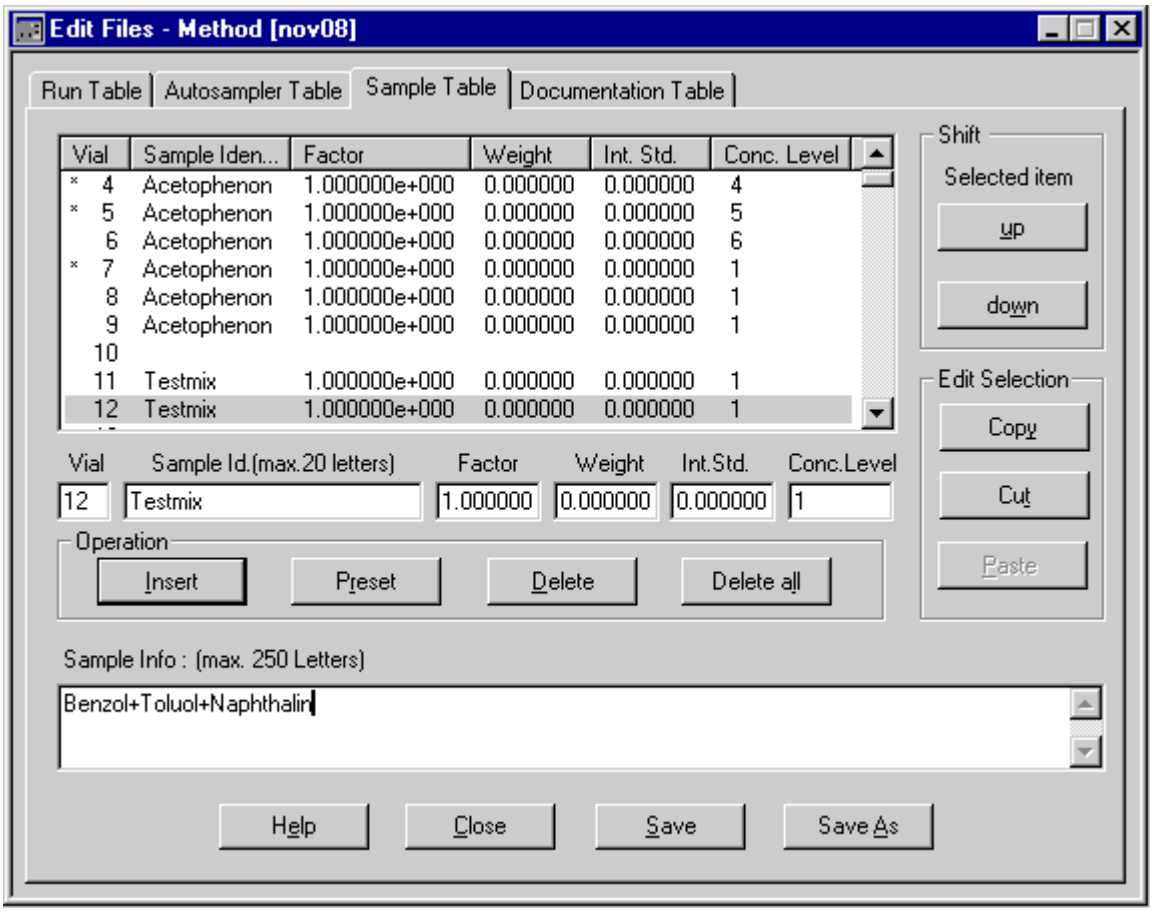
An empty table appears if the autosampler table is selected in a new file. The cursor is blinking in the first field of the entry bar. Entering

1 TAB TAB 2 TAB 20 TAB 8 TAB TAB new TAB Downwards arrow Short32.rpf TAB new TAB

and clicking *Insert* accomplish the execution of 2 successive injections from vial 1 of 20 µl. These injections are carried out immediately once the preparation of the autosampler is completed. The pump starts pumping the solvent mixture as specified in the LC procedure file New. The chromatograms are stored under the name specified in the Run Table, which is extended by 0011 and 0012, and are printed out using the short32 report print template.

4.1.1.3 Edit Files - Method - Sample-Table

When *Sample Table* is clicked a table appears in which parameters with the following function can be entered:



Inj., Vial Injection number or vial number when using an autosampler, entries from 1 to 499.

Sample Id. The name (up to 20 characters) entered here appears on the chromatogram print out behind Sample Identifier. It is stored in the raw data file (.SLI) with the chromatographic data.

The following parameters are used in quantitative evaluations:

Factor. This factor is used as a dilution factor in a quantitative evaluations with the internal or external standard methods. The result of the quantitative calculation is multiplied with this factor.

Weight. Total quantity of the injected sample. In a quantitative calculation the quantities found for the peaks to be calculated are also calculated as a percentage of this total sample weight.

The quantity must be entered here in the same units as used for the standards in the Calculation-File (section 4.1.3.2).

Int. Std. The quantity of the internal standard. If this differs from the quantity used in the calibration, this new value can be entered here. Entries must be in the same units as those of the Calculation-File.

The parameters *Factor*, *Weight* and *Int. Std.* are saved together with the chromatogram, they are used in later calculations, but also can be changed before carrying out a calculation.

Conc.Level. Number of the calibration sample with defined standard concentration for Multi-Level-Calibrations.

The level number entered here must correlate with the concentration defined under the same level number in the peak table of the calculation file (cp. p- 4.1-26).

Sample Info. Additional sample information can be entered here for each line in the table.

Entry of parameters is described in 4.1.1.2.

Several lines of the table can be marked by using the mouse. The *Up* and *Down* key in the *Shift - Selected items* box are used to move the lines up- or downwards. The *Copy*, *Cut* und *Paste* key in the *Edit Selection* box can be used to copy, cut or paste marked lines.

Insert inserts the edit line into the table. *Delete* deletes a marked line. *Delete all* deletes all lines.

The various entries in the Sample Table are used during an analysis when carrying out a number of sequential injections. When a method is recalled later and started, the Sample Table commences again at injection 1 or the first vial, respectively.

When using an autosampler the SampleTable contains the correlation of the sample name (identifier) to the vials.

Before starting the analysis a defined line number of the Sample Table or the Autosampler Table can be specified as start line (chapter 4.2).

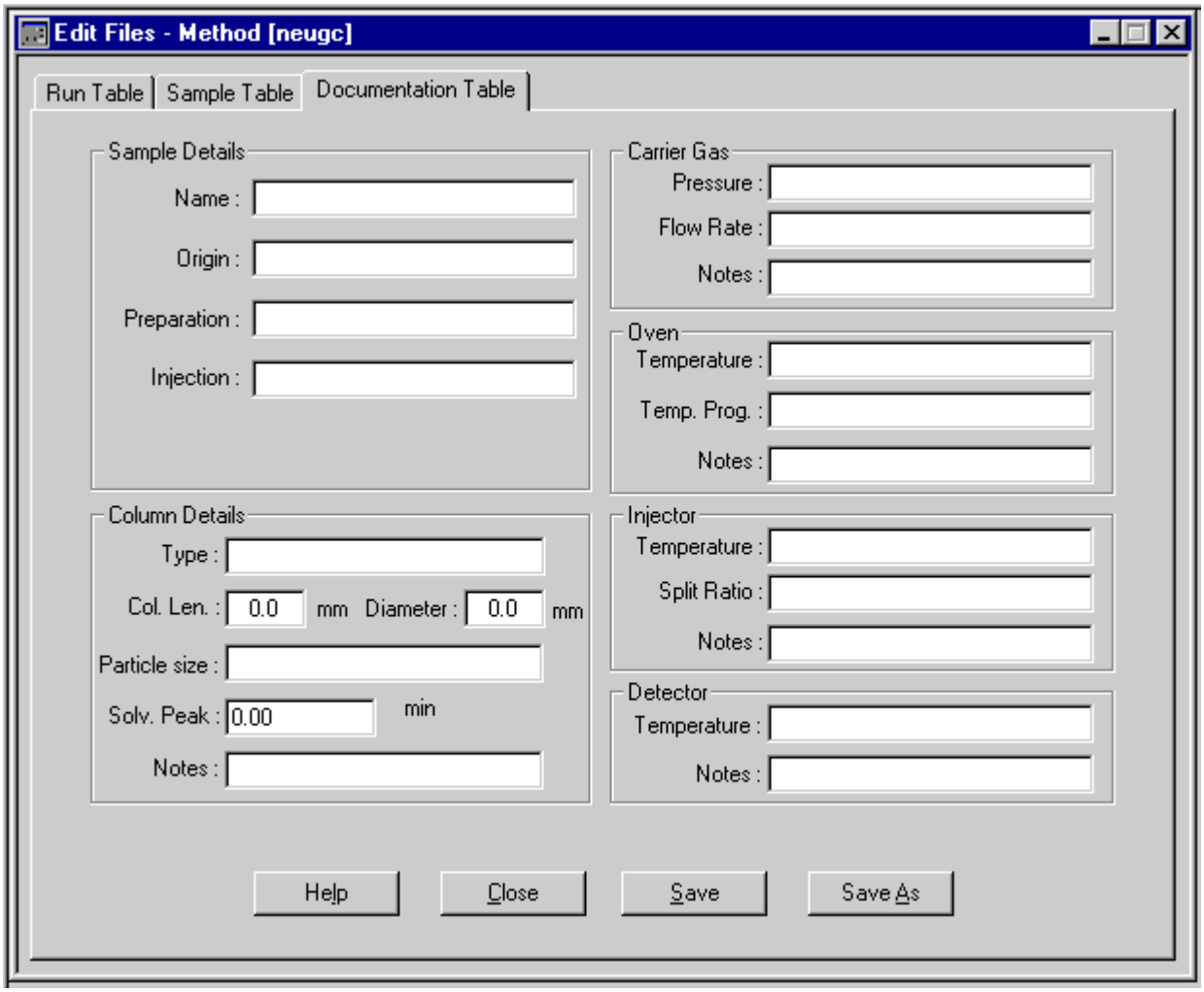
Vials which are already analysed are marked:

- Chromatogram is recorded (.SLI and .RPT-files exist).
- Appears when using an autosampler and injecting several times out of one vial, where the injections are not yet finished.

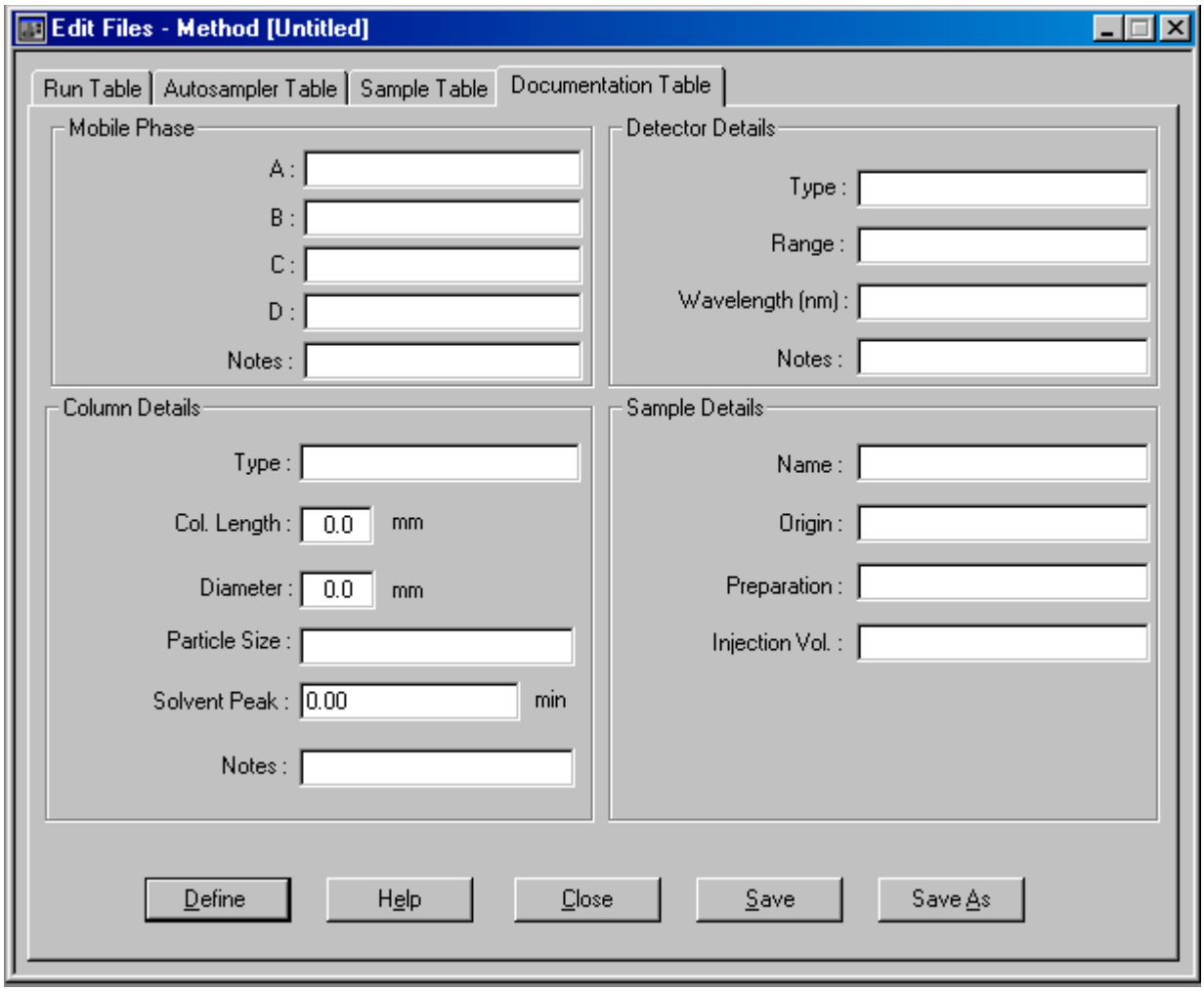
4.1.1.4 Edit Files - Method - Documentation-Table

Access this table by clicking into Documentation Table. Here you can make notes about the sample preparation, the column, the eluent and the detector being used for the method. If in the Run Table Mode= GC is chosen GC specific entries as shown below can be made here.

TAB accesses the next field, Shift+TAB accesses the previous field.



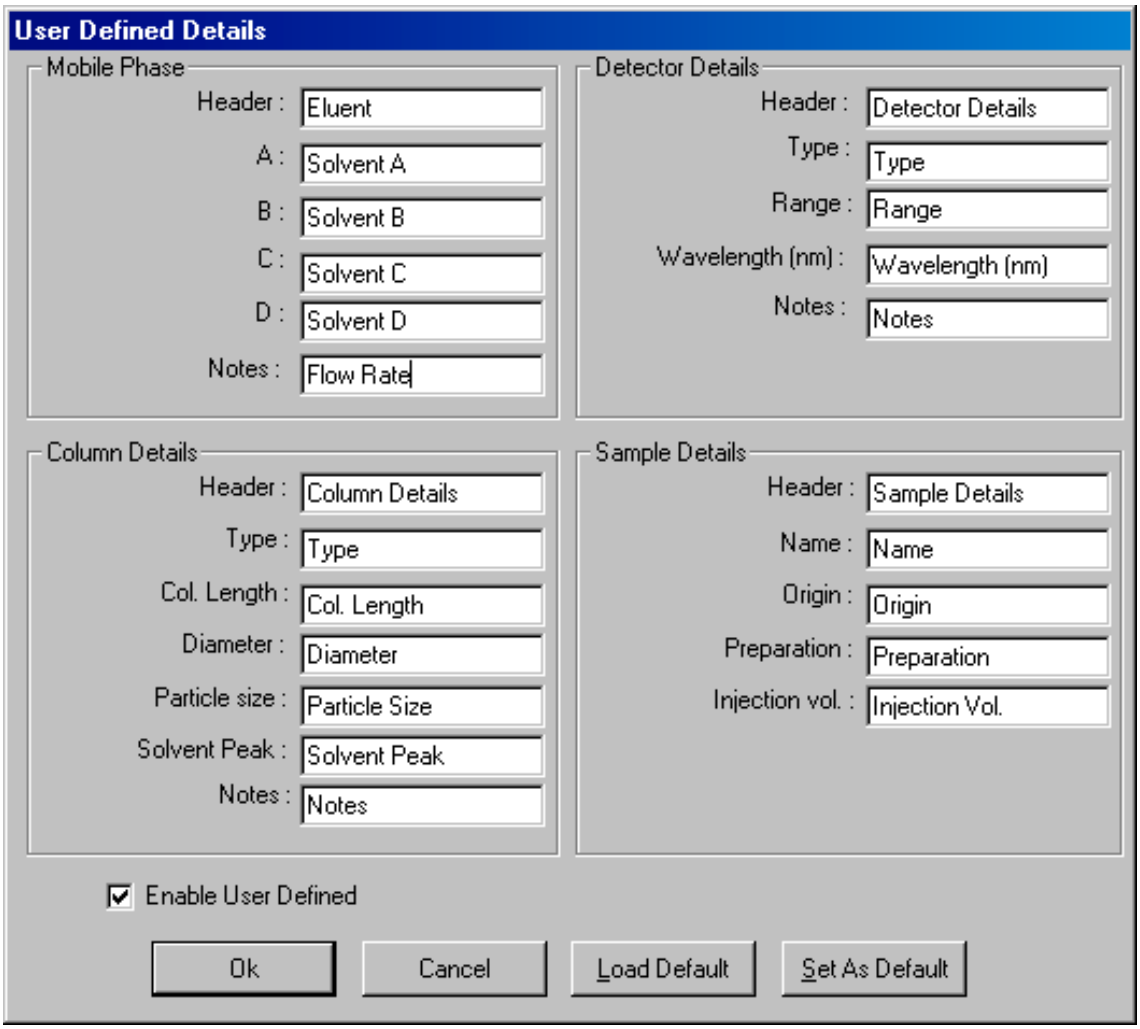
If Mode=HPLC is chosen, the following entry boxes appear:



The entries under *Column Details* against *Column Length* and *Solvent Peak* are used for calculation of column coefficients. For the calculation the preset column length is 125.00 mm, the solvent peak (or dead time, retention time of a an unretended solute) is set to 1.00 min. These values can be changed as required. For more details about column coefficients see chapter 5.2.6.

The *Define* key opens the *User defined details* box, where the descriptions of the items, which appear **in front** of the entry boxes, can be changed.

In the following example the descriptions in the *Mobile Phase* box were changed. The box now shows the head line Eluent and the box texts Solvent A, Solvent B, Solvent C, Solvent D and Flow Rate. The user defined descriptions can be saved in the Chrst32.INI file in the chapter [Document Table] by using the *Set as Default* key. When creating a new method file, the ChromStar descriptions are then overwritten by using the *Load Default* key .



4.1.2 Edit Files - Data-Handling

The data handling file (extension = INT) is necessary for defining data-acquisition and integration parameters. Recording a chromatogram requires a data handling file; one data handling file can be used for recording a number of chromatograms.

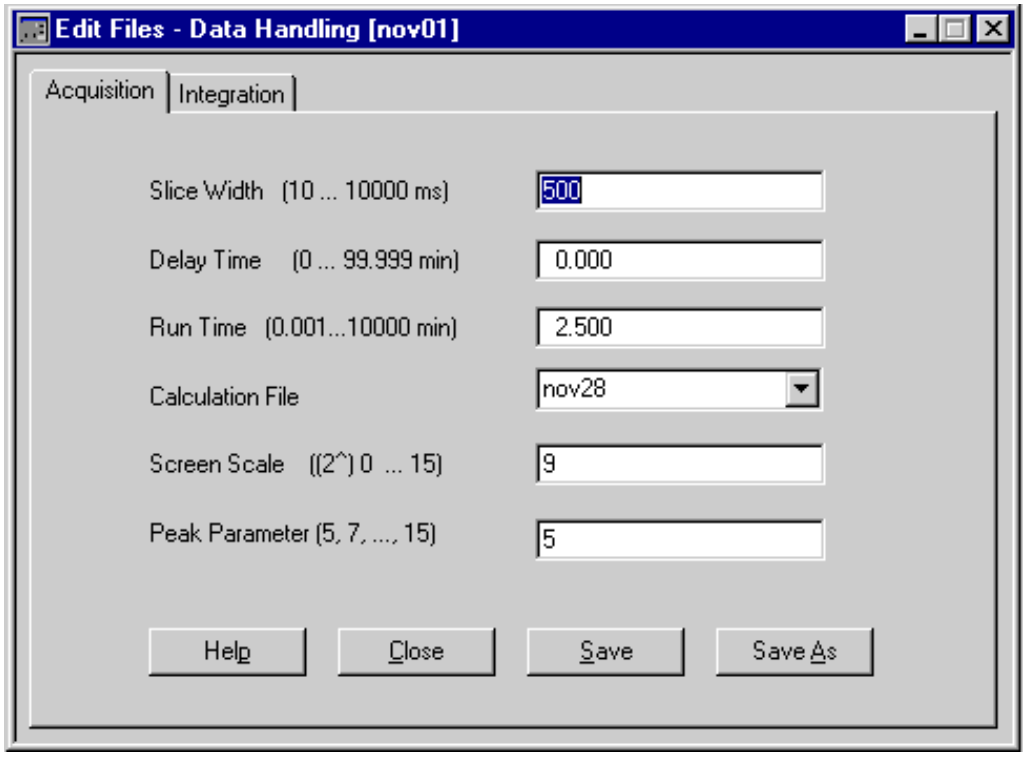
After Data-Handling has been selected from the submenu of Chrom.Files or using the button, the dialog box Open Data Handling File appears for entry of a name; it is used exactly the same as the Open Method File field (Chapter 4.1.1).

After entry of the file name the menu bar appears with the points

File Window Help

The data handling file has two pages. The first (Acquisition Page) contains the parameters for data recording, the second (Integration Page) contains the integration parameters which can be programmed on a run time basis.

4.1.2.1 Edit Files - Data-Handling - Acquisition Page



The parameters of this page have the following functions:

Slice Width (ms). This parameter defines the time in ms (range 10 to 9990 ms in steps of 10 ms) before each next data point is recorded (data-acquisition rate).

Delay Time (min). During this period (entries from 0 to 99.999 minutes, in steps of 0.001; default value 0.1) after the start of an analysis chromatographic data are recorded and stored but not integrated. This parameter can be used to prevent integration of solvent peaks which appear at the start of a run.

Data acquired during the delay time can be integrated later in a reintegration run by defining Delay Time = 0.

Run Time (min). During this period (entries from 0.01 to 10000 minutes, in steps of 0.001; default value 60.000 with a slice width of 500 msec) the chromatographic data of a run are acquired. If this period is longer than that defined under Run in the method file (using the autosampler), the data acquisition will stop when the time entered in the Autosampler-Table is passed.

If a shorter Run Time is entered than in the Autosampler-Table the data acquisition ends after the time specified here in the Data-Handling-File.

Calculation File. The name of a calculation file (default value = name of the data handling file) can be defined here. It is used to perform quantitative calculations with the integrated peaks data.

If no name is defined or if the calculation file defined does not exist, a percentage calculation is made.

Screen Scale (2^). This parameter defines the scale of the y- axis on the screen (0 to 15). The value 10 shows the detector signal from 0 to 313 mV, 9 shows the range 0 to 156 mV, 8 the half value again etc.

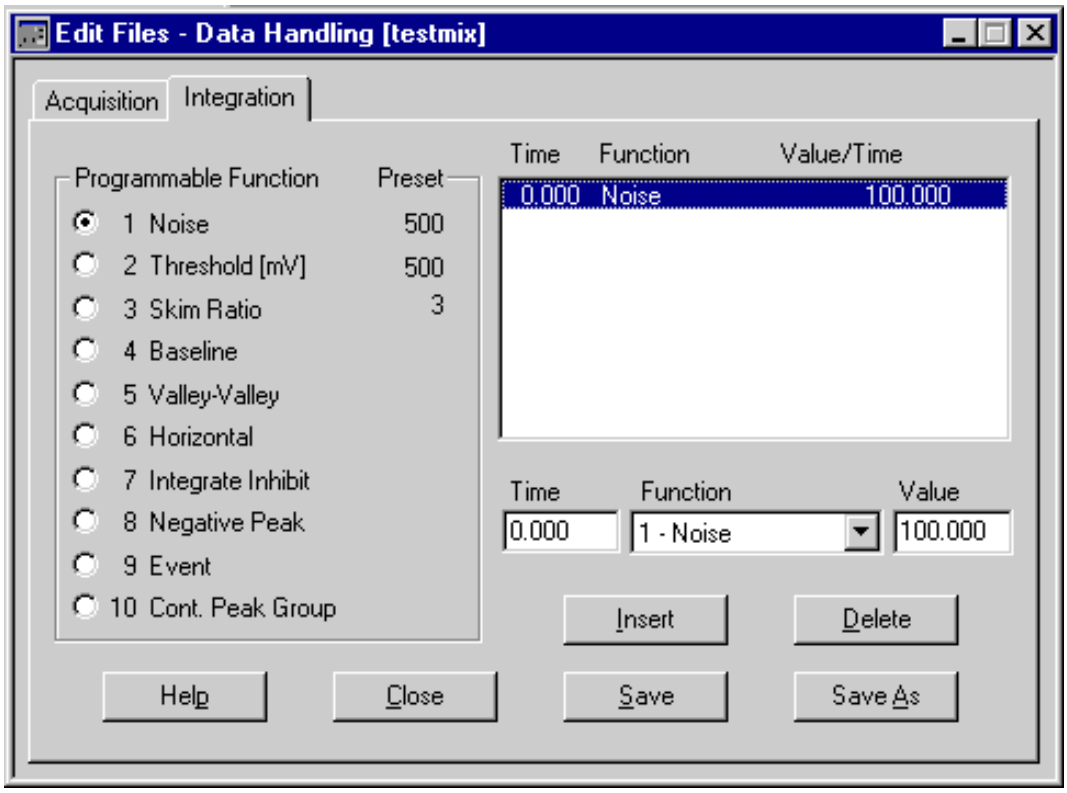
Peak Parameter. This parameter defines the number of successive data points to be used for averaging the slope of the detector signal function (lowest value 5, highest value 15, odd values). The slope is checked for a certain number of data points for the determination of peak start and peak end.

After accessing the data handling file the first parameter of the acquisition page appears on a black background and can be now be edited. TAB accesses the next box down which now has a black background; Shift + TAB accesses the previous box. The entry fields can also be accessed with the mouse cursor, after over striking with a depressed mouse button a black background appears and now a new entry can be made.

Clicking the check boxes switches between selecting and de-selecting.

4.1.2.2 Edit Files - Data-Handling - Integration Page

The Integration Page appears after clicking the corresponding index card.



The parameters of this page can be programmed on a run time basis and have the following function:

1 Noise. This parameter controls the recognition of peak start and peak end. The value of the noise (Microvolt/second) defines the maximum slope of the detector signal which is not yet recognised as peak start. The noise value can be set between 0.001 and 10000 and can be changed at any run time.

2 Threshold (mV). With Threshold a height of the detector signal is defined above which no new baseline is allowed to be set during a slope of the signal higher than the noise value. This parameter is used to prevent a broad valley between two peaks being evaluated as a baseline. In the same way, the parameter Threshold ensures that a peak which has had its top cut off (overloading of the detector) is correctly integrated and is not treated as a double base line offset. Its value can be between 1 and 1000 and can be modified at any runtime.

3 Skim Ratio. This parameter compares the ratio of the heights of two adjacent peaks to the height of their common valley. It determines whether they should be separated by a perpendicular line or whether the second peak is to be regarded as "riding" on the first in which case its baseline runs exponential from the valley between the peaks to the peak end. Its value can be set from 0 to 20.

4 Baseline. This parameter allows a baseline for the integration to be set manually at a defined time during the data acquisition - for example after a disturbance of the baseline caused by a change of solvents, of the flow rate or of the wave length.

Further explanations concerning Noise, Skim Ratio and Baseline can be found in chapter 5.2.2.

Attention! Be careful with the definition of the baseline when the retention times are unreliable. If the baseline is set during a peak the area and height of the peak will be incorrectly calculated.

5 Valley-Valley. This parameter defines a time window during the data acquisition in which a baseline is plotted between all adjacent valleys regardless of the Threshold and Skim ratio values set. This allows a better integration of peaks which appear in the chromatogram upon a broad and unresolved peak. The sharp rise of the baseline caused by column bleeding in temperature-programmed GC can be compensated for here.

6 Horizontal. This parameter defines a time window during the data acquisition in which the baseline is plotted horizontally from the height of the signal at the beginning of the time window until the end of the time window. This parameter serves to generate a horizontal baseline over a negative peak. If this were not done the baseline would be incorrectly calculated.

7 Integrate Inhibit. This parameter defines a time window during the data acquisition in which no integration takes place. During this period the chromatographic data are recorded and stored; the baseline setting is continued at the time window borders.This parameter allows the integration to be interrupted when the peaks are insignificant or when the detector signal alters as a result of a change of wavelength.

8 Negative Peak. This parameter defines a time window during the data acquisition in which a negative peak is regarded as positive and integrated accordingly.

9 Event. The output channels of the Event Box LC 427 can be time-switched On and Off with the help of this function (0 = Off 1 = On). Up to 8 events per channel can be switched.

10 Continous Peakgroup. This parameter defines a time window in which a peaks group is evaluated as one peak.

All parameters are programmable on a time basis. Enter the start time against Time in the time table, the number of the requisite function against Function and the end time against Value or the desired value for the functions 1-3.

Program as follows:

Click the Time box at the right in the entry line with the left mouse button and enter the desired time (in units of 0.001 min from 0 to the time defined against Run Time on the Acquisition Page). Jump to the next box with TAB. Select the desired function by entering its number as given in the table at the left or click the function in the table itself. Jump to the last box with TAB and enter the value for the defined function - for the functions 4 to 8 a time in minutes. For the Event function programming against Value is done by entering the event number, space, and on (1) or off (0). The line is inserted into the table above by clicking *Insert*. If a line needs to be deleted from the

table click it with the mouse. The line now appears in the table on a black background and in the edit line, it can now be deleted with *Delete*.

Entries in the table are sorted according to their time.

Attention! A total of 40 values of the same parameter and 75 values of different parameters can be defined.

After creating the two pages of a data handling file it must be stored before carrying out an analysis. This is done with one of the appropriate submenu points of the menu point *File* or using the screen keys. It is not essential to fill out the second page. The default values are used if no entries have been made.

Using ***File*** and ***Open*** or the button  a file can be selected and opened.

Customize Function Names allows you to change the descriptions of the programmable functions (1 – 10) and to save these in CHRST32.INI in the section [Edit Files].

Atention! The new descriptions appear in all Data Handling files.

The original descriptions reappear on deleting the entries in CHRST32.INI in [Edit Files].

Using ***File*** and ***Close*** or the button  a file is closed.

4.1.3 Edit Files - Calculation

The menu point ***Calculation*** allows the calculation file (file extension.CAL) necessary for calibration and quantitative calculations to be created.

After clicking ***Calculation*** in the ***Chrom.Files*** submenu or using the button, a file name must be selected as described in 4.1.1 or a new name entered. After selection, the following points appear in the menu bar

File Window Help

and the first page of the calculation file appears, the parameters of which will now be described.

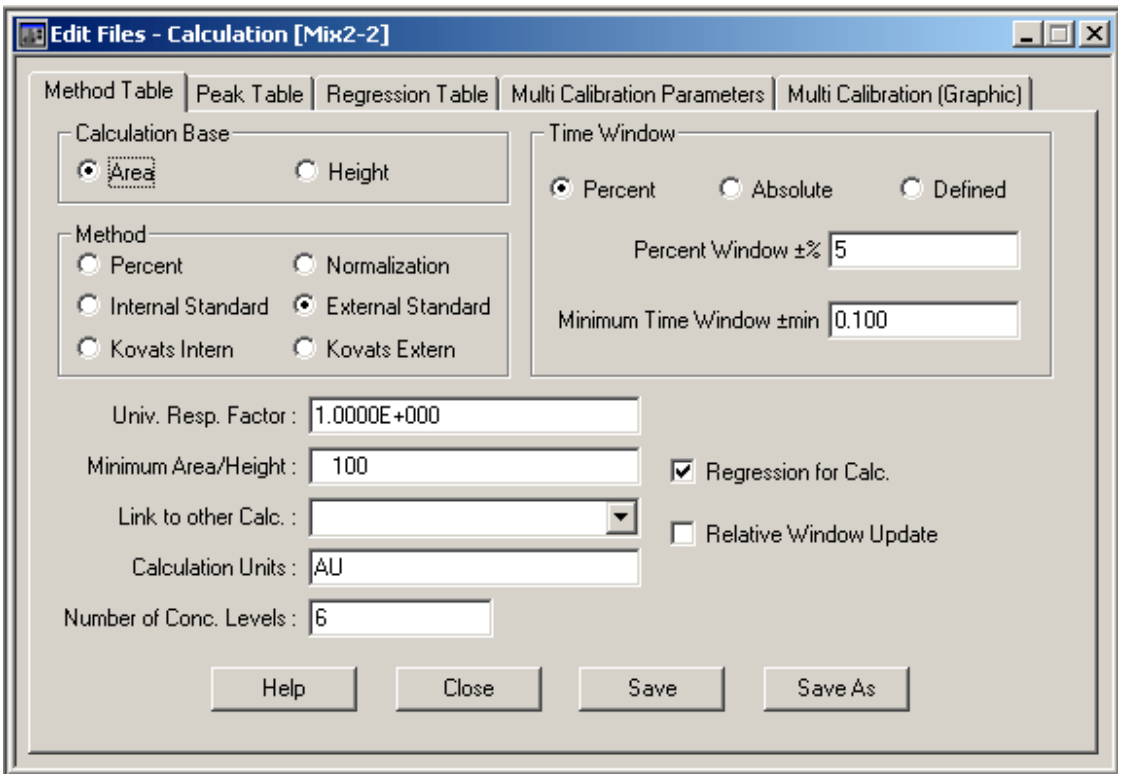
The calculation file can be stored with ***File*** and ***Save*** or with ***File*** and ***Save As***, in the last way under a new name, or by using the screen keys. After changing a file and clicking ***File*** and ***New, Open...*** or ***Close*** a question is displayed asking whether the changed file has to be save or not.

With ***File*** and ***Open*** or the button  another file can be selected and opened.

With ***File*** and ***Close*** or with the button  the Edit Files - Calculation window is closed.

4.1.3.1 Edit Files - Calculation - Method-Table

On the first page of the Calculation-File parameters are to be entered concerning the type of calculation, the type of time window and a second calculation file if required can be specified.



The following parameters can be defined:

In the **Calculation base** box:

Area/Height. This parameter defines whether areas or heights are to be used in the calculation. Click the option required.

In the **Method** box:

Percent/Normalization/Internal Standard/External Standard. This parameter defines the type of calculation to be carried out (cp 5.3.2 description of calculation methods and formulae).

The default method is percentage, this is carried out when the calculation file specified in the data handling file is not found or when no calculation file is specified. In the percentage method the results list shows for each peak the retention time, peak start and end time, peak area and height and area respectively height in percent.

When using the percent method with entries in the peak table (cp. p. 4.1-24), the percentages of all peaks are calculated, for the peaks in the peak table the response factors are used in the calculation, the peak names are annotated if any names are entered.

In the normalization method the results list shows the retention time, the area and height of all peaks and also the area percent and names of the peaks which are defined on the second page (Peak Table).

Select Internal Standard when this method is to be used for the evaluation. A calculation of areas and heights of all peaks takes place as well as a quantitative calculation of all peaks defined on the next page. On that page (peak table) the internal standard peak must be marked against Code with IS.

The External Standard leads to a calculation of the areas and heights of all the peaks as well as a quantitative calculation of all peaks defined on the next page.

Select the desired method by clicking the appropriate box.

Kovats Intern/Kovats Extern. Kovats Indices can be calculated in GC-chromatograms after internal or external calibration. The standard values are entered on the second page against Kovats Ind. (for details see chapter 5.2.5)

Univ.Resp.Factor. Universal response factor, only valid, when Method=Percent and entries in the Peak Table are made. The universal response factor is used for all peaks in the Peak Table.

Minimum area/height. This parameter (0 to 1000000; default value 100) defines the level of peak height or area obtained from an integration or reintegration below which a peak is excluded from the quantitative calculation.

Attention! When peak heights are used in the calculation the value entered here must be considerably lower!

Link to other Calc. In this field the name of a Calculation-File can be entered (8 characters, default is blank) for a further quantitative calculation of the current run. This can be used to perform various types of calculation e.g. firstly calculation with peak areas and then with peak heights. During a calibration run it is nor possible to carry out a further calculation with this parameter.

The Link parameter can be used to carry out calculations with more than one internal standard. For each internal standard and (only) for the peaks evaluated by using this standard a calculation file must be generated.. During calibration there must be no entry against the Link parameter, the calculation method is Internal Standard. For the calculation the name of the second (and more if necessary) calculation file is entered against the Link parameter. The results are listed in one table.

Calculation Units. This parameter (8 characters) defines the unit of measurement in which the results are specified. This unit is printed as the column heading for the results table.

Number of Conc. Level. Number of different calibration standards (max. 15) which are used in the Multi-Level-Calibration.

Regression for Calculation. This parameter defines whether the regression coefficients generated from a previous multi-level calibration (see sections 4.4.2.3, 5.3.2.5 and 5.3.2.6) and stored on page 3 of the calculation file are to be employed in a quantitative calculation. Clicking the box switches between on and off.

Relative Window Update. This parameter defines whether the time window used for defining peaks or peak groups is to be updated (shifted) at the end of each run. The reference peak is specified as RF against Code on the second page of the calculation file. An update does not take place if no mark is clicked into the box or if no reference peak is defined in page 2 of the calculation file.

Attention: Do not use with calibrations!

In the **Time Window** box:

Percent/Absolute/Defined. This parameter (default value: percent) defines the type of time window calculation to be performed in identifying peaks.

If the preset type (time window = the retention time ± time in percent) is not required click one of the following boxes (Absolute: time window = the retention time ± time in min. Defined: each peak on the next page has a time window with precisely start and end times).

The last two entry fields of this page and the column headings of the table on page 2 vary according to which type of time window calculation has been selected. After selecting the time window option Absolute the last parameter of this field is cancelled. Selecting the option Defined means that the last two parameters of this field are cancelled.

Percent Window ± %. If the time window option Percent has been selected this parameter (integer in the range 1 to 99; default value 5%) defines the percentage size of the time window for all peaks. Different time window sizes can be entered for individual peaks on page 2 of the calculation file.

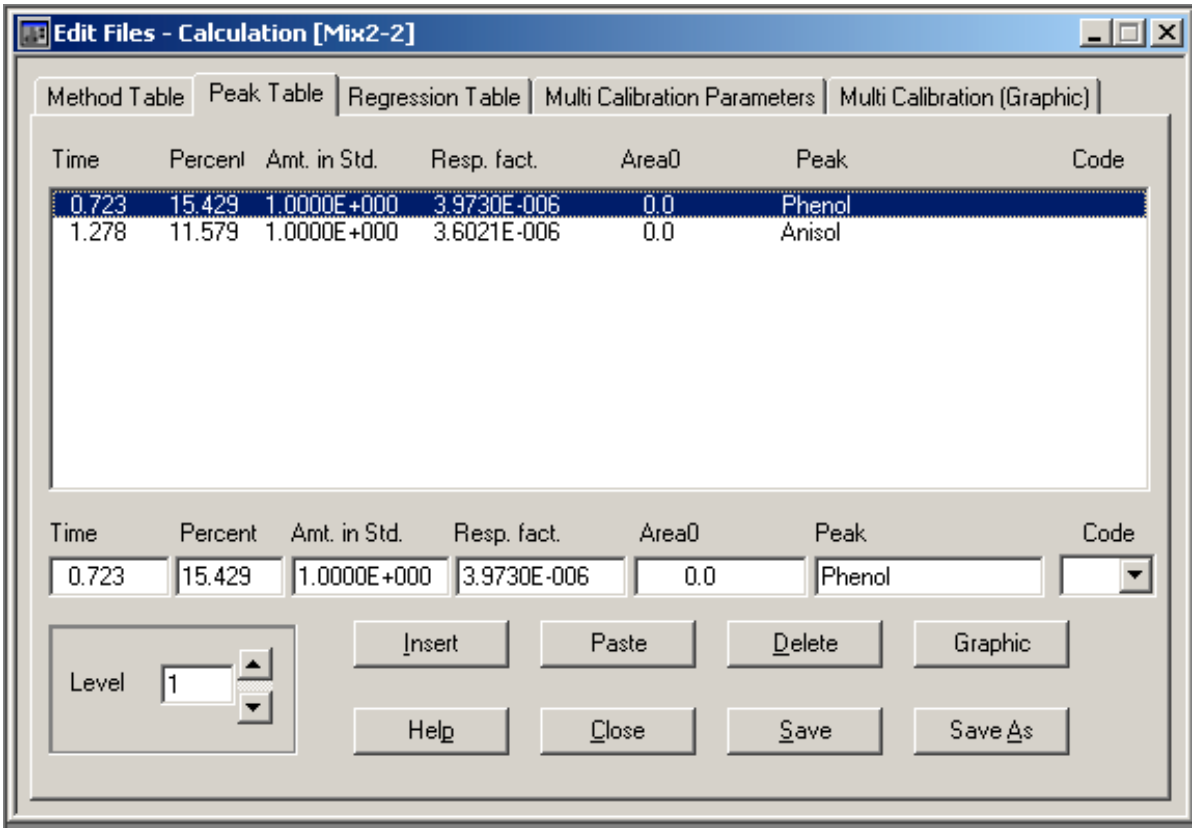
Absolute Window ± min. If the Absolute option has been selected this parameter (0.001 to 9.999 minutes; default value 0.05) defines the time window in minutes for all peaks. Here also it is possible to define different windows for individual peaks on page 2.

Minimum Time Window ± min. If the Percent option has been selected this parameter (0.001 to 9.999 minutes; default value 0.01) defines an additional minimum absolute time window. If the percentage time window calculated for a specified retention time leads to a value lower than that defined here, the time window is defined as being retention time ± minimum time window.

The parameter values can either be accessed with the TAB key or Shift+TAB, the entries appear on a black background. The same happens after striking over an entry box with the cursor while the left mouse button is depressed. A new value can now be entered. The next box to be edited can again be accessed with the mouse or TAB.

4.1.3.2 Edit Files - Calculation - Peak-Table

The parameters of the second page of the calculation file are used to define peaks or groups of peaks and their various data (e.g. quantity, response factor, name or code). On this page data are entered into a table which is automatically sorted chronologically when new peaks are entered. The column headings of this table depend on the method previously chosen to define time windows.



The following columns are used:

Time. In this column the retention time (0.001 to 999.999 min) is entered for each peak or group of peaks. This column is used for chronologically resorting of the table. A new entry at an existing time causes the original values to be overwritten.
The time entered in this column should be as close as possible to the time of the peak maximum or in the case of a peak group to the time in the centre of the group. If defined time windows are being used this column is cancelled; time values are then entered against Time 1 and Time 2 (see below).

Percent. This column is available, if the percent time window has been selected, to enter another percent time window for each individual peak retention time than that defined on page 1.
The range limits of the entry are the same as those of Percent Window ± %.

± min. If the Absolute time window has been selected the column heading reads ± min. A different time window than the absolute time window defined on page 1 can be entered here for each peak.

Time 1. This parameter (0.001 to 999.999 min) defines the start time of the time window for an individual peak.

Time 2. This parameter (0.001 to 999.999 min) defines the end time of the time window for an individual peak. This time must be greater than Time 1.

The following columns are valid for all types of time windows.

Amt.in Std. In this column a known amount (1.0000E-009 to 1.0000E+009 in the unit of measurement defined under Calculation Units - default value 1) can be entered of a component of a calibration mixture with the retention time specified in column 1. Herewith the response factor (Resp. Fact. cp. next column) for this peak can be calculated after a calibration run.

Resp. Fact. This column contains the response factor (1.0000E-009 to 1.0000E+009 - default value 1) for a specified peak or peak group. The default value of 1 is used during a calibration run.
After a calibration run the response factors are automatically transferred to or updated in the calculation file.

Area0. Here the user can enter a peak area obtained from a calibration run to use it for a Standard-Elevation-Method. The area entered here is subtracted from the total peak area before the unknown amount is calculated.

The entry Recovery=Yes in the CHRST32.INI file in the chapter [Calibration] changes the column header to Recovery (%) and enables a quantitative evaluation under respect of a recovery rate. The recovery rate is entered in %. It can be different for the different peaks. The default value is 100 for a recovery of 100%.

Peak. In this column a peak name of up to 14 characters can be entered which belongs to the retention time at the start of a line. The name is printed in the results table behind the corresponding retention time.

Code. In this column a one or two character code can be assigned to a specified peak. The following codes may be used:

IS - This code defines a peak which is to be used as the internal standard. If more than one peak is assigned this code only the first one is used as the internal standard.

RF - This codes defines a peak which is to be used to update the time windows after each run. It is necessary that the parameter Rel. Window Update on page 1 of the calculation file is switched on.

G - For peak grouping a peak time window is marked with G. The areas of all peaks appearing in this time window are added. In this calculation the retention time is the average of the retention times of the peaks in the time window. In the chromatogram only the centre of the peak group is marked with its retention time or peak number. In the report the peak group appears on only one line - identified as G - for a group of peaks. Several peak windows can be marked with G in a chromatogram which leads to an equivalent number of peak groups being calculated.

S + Number - For peak summing various non-adjacent peaks can be marked which are added in the evaluation. A number of different peak sums can be defined with S1, S2, S3 etc. The retention time and the name of the first peak in the current sum are used. The peak sums appear in the report in one line - identified by S + Number. The area is the sum of all the peak areas of the peak windows identified as S + the same number.

The parameter Minimum Time Window does not work when peak groups or sums are being computed (further explanation see section 5.2.4).

After clicking the *Peak Table* index card the table appears with all entered values and an entry line. The cursor blinks in the first box of the entry line. After editing the next box is accessed with TAB or by clicking in with the mouse. The line is inserted into the table and sorted chronologically by clicking *Insert*.

Retention times, which are part of the peak data in existing Report-Files (file extension .RPT), can also be imported into the peak table with the ***Import Peaks...*** command from the menu point ***File***. Selecting of a report file and clicking OK copies the retention times into the peak table. Clicking the *Import Peaks...* command again deletes the imported data from the peak table.

If a line in the table is to be deleted or changed, click it with the mouse. It then appears in the entry line below. It can now be changed using TAB and entering the desired parameter value. It is entered in the table again by using *Insert*, or deleted by clicking *Delete*. If more than 11 lines are required the scroll bar is displayed at the right side allowing you to scroll through the table.

The number of the next calibration solution can be selected by clicking in the scroll bar of the box down left behind **LEVEL** or by entering its number. Here after only the amounts of the components in the calibration samples can be entered in the entry line against Amt.in Std. The user must enter this for each peak in the calibration solution.

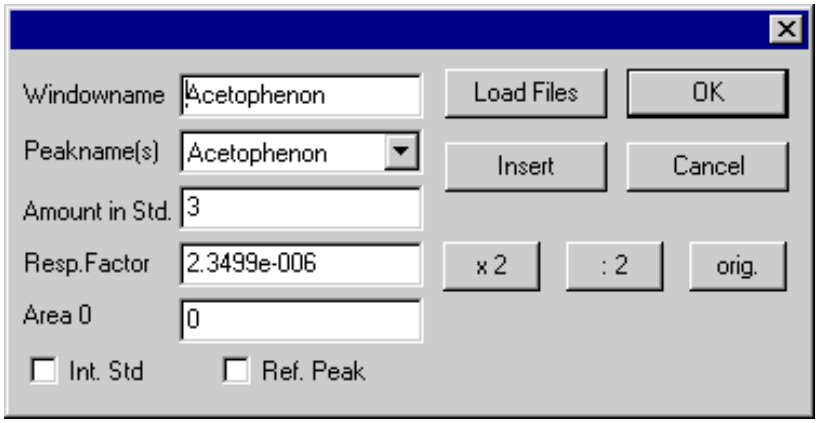
The ***Paste*** key transfers the content of the clipboard into the table. Previously, the relevant peaks must be selected in the Peak Report Table and copied into the clipboard by using the ***Copy*** key in *Results* in *Reprocess*, *Reconstruction*, *Reintegration*, or *Manual Integration* (cp. p. 4.4-8, 4.4-12, 4.4-18).

If more than one peak is in a peak window, a peak can be selected for the calculation by entering in the chrst32.ini file:

[Calibration]

PeakDetection=1	default value (all peaks in the window are evaluated)
=2	the largest peak in the window is evaluated
=3	the peak in the middle of the window is evaluated
=4	the first peak in the window is evaluated.

The ***Graphic*** key allows graphic peak window definition. After clicking ***Graphic*** a file select box is opened where chromatograms can be chosen. These appear in an overlay mode representation. An entry box appears where detailed information about the peak windows can be made.



A peak window is defined by moving the mouse with pressed left button from the peak start to the peak end, either to the left or to the right. This area appears high-lighted.

In the entry box the following entries can be made:

Windowname: Peakname, will be added under *Name* in the Peak Table.

Peakname(s): If a calculation with peak names was carried out earlier, the name used appears here. If more than one peak name is found in the window, all the other names appear by clicking into the downwards arrow. By clicking into one of these names the name will be transferred into the box after *Peakname*. It will be inserted in the *Windowname* box by using *Insert*.

Amount in Std.: Amount in the standard solution, corresponds to *Amt. in Std*. in the peak table and will be inserted there.

Resp. factor: Response factor, corresponds to the *Resp. Fact*. in the peak table and will be inserted there.

Area 0: corresponds to the same item in the peak table.

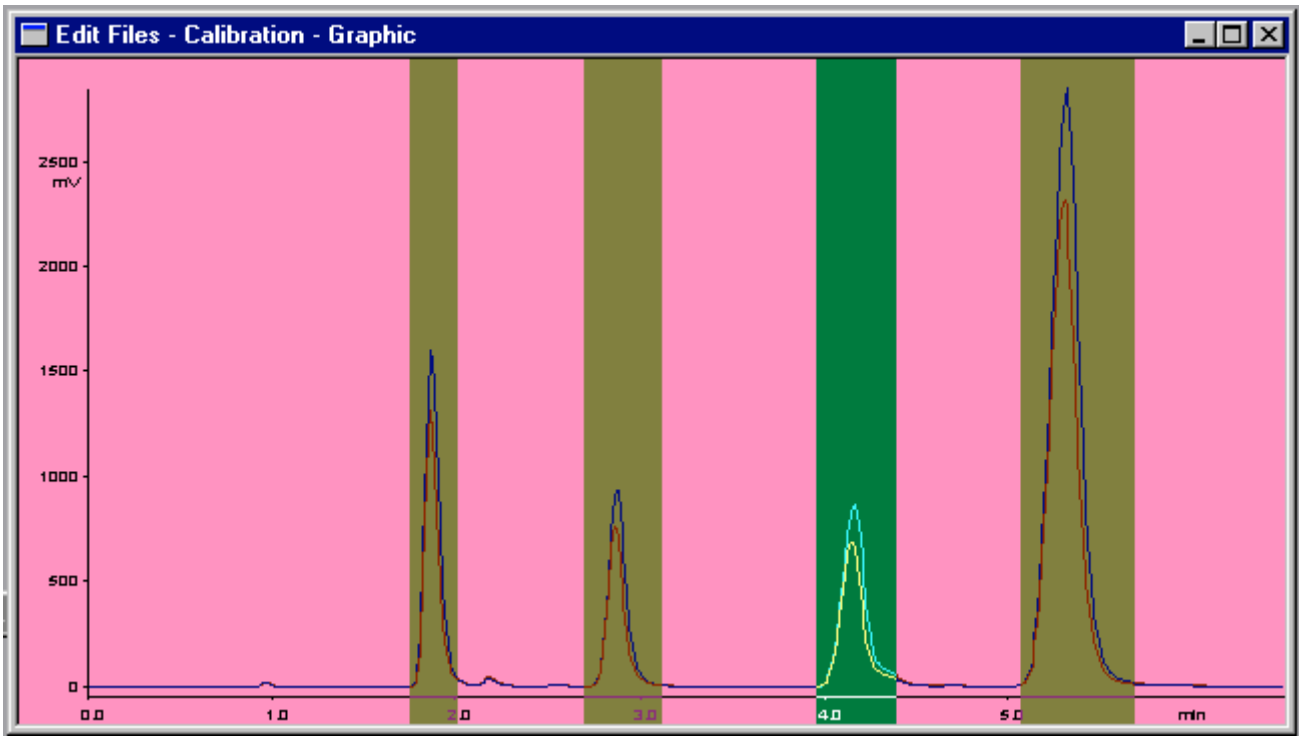
By clicking into *Int.Std.* or *Ref.Peak* one of the peaks can be specified as internal standard or reference peak (do not use with calibrations!).

x 2, : 2, orig. : multiplying or dividing the retention time by 2 or re-establishing the original value. These entries can be made in a box which appears by clicking the right mouse key in a peak or via menu point ***Zoom***.

By using ***Edit, Delete*** or the *Delete* key or  a peak window can be deleted.

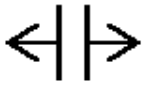
After using **x2** a scroll bar appears at the bottom of the chromatogram, tus enabling all ranges of the chromatogram to be displayed.

As described above more than one peak can be defined.



When dragging the mouse along the chromatogram the cursor shows different shape.

Peak start and peak end can be moved by pressing the left mouse button and moving the mouse to the right or to the left.

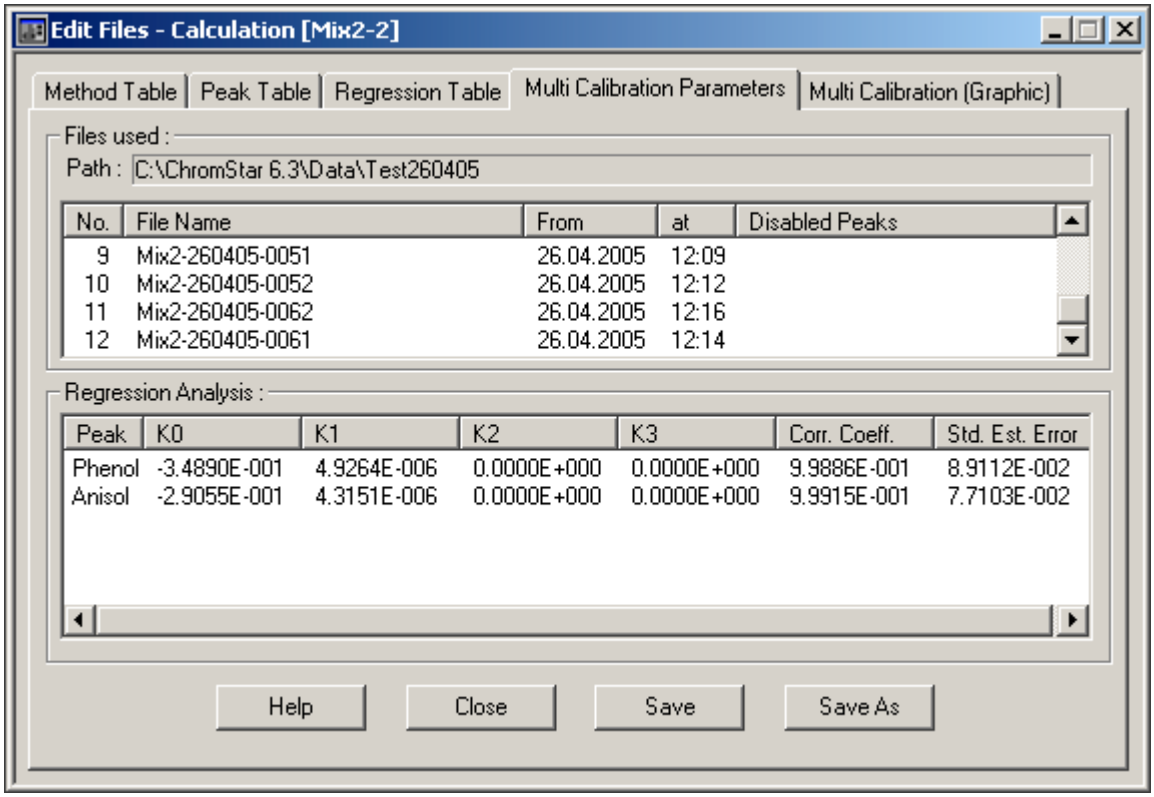


The whole range of the peak window can be moved by pressing the left mouse botton and moving the mouse.

Load Files leads back to the file select box.
With OK or ***File, Return*** the determined peak windows are transferred into the peak table. The peak table reappears with the new entries.
With *Cancel* the procedure is aborted. The peak table reappears.

4.1.3.3 Edit Files - Calculation - Regression-Table + Multi Calibration

The Regression Table of the calculation file contains the function coefficients of the 1st, 2nd, or 3rd order regression analysis of a multi-level calibration for the peaks defined in the peak table of the calculation file or, respectively, under K1 the average value of the response factors after multi calibration with the Result-Files of a multiple injected calibration sample.



After determination of the calibration functions K0, K1, K2, and K3 are the regression coefficients of the equation

$$y = K3 * x^3 + K2 * x^2 + K1 * x + K0$$

where y = amount of a substance, x = peak area or peak height of this substance.
These coefficients are generated in a multi-level calibration (cp. section 4.4.2.3) and they cannot be altered manually.

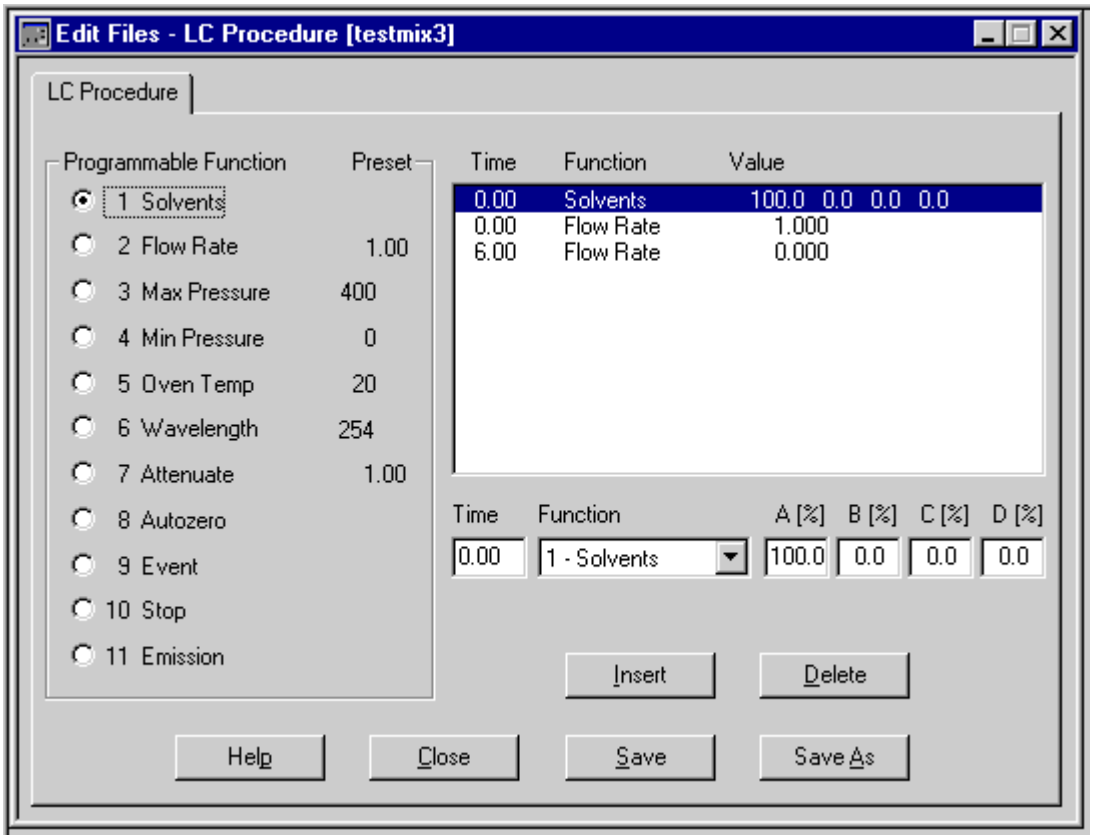
The ***Multi Calibration Parameters*** index card of the calculation file shows the chromatogram names of the standard solutions used in the Multi calibration. The table beneath shows the regression analysis of averaging the response factors or of the multi-level calibration, respectively.

The ***Multi Calibration (Graphic)*** index card shows a grafic display of the multi calibration. By clicking with the right mouse key in the grafic display a peak list appears, in which another peak to be displayed can be chosen.

In ***Reprocess, Calculations*** a calculation file with a multi calibration can be loaded and edited via the menu procedures ***File*** and ***Load Multi Calibration***.

4.1.4 Edit Files - LC-Procedure

The LC procedure file is used for controlling an HPLC pump if this is equipped with an appropriate interface. Clicking the menu points *Chrom. Files* and *LC-Procedure* in the Edit Files window or using the pro button and entering a file name as described in 4.1.1 displays the file on to the screen.



In this file (file extension .PRO) the following parameters can be time-programmed:

1 Solvent. This function defines the solvent composition at a certain time. Entered here is the percentage proportion (from 0.0 to 100) of the solvents A, B, and C separated by a space. The proportion of solvent D is automatically calculated.
Attention: An LC Procedure must contain a solvent programmed at time 0.
The programming of a gradient (see section 5.1.2) requires only the specification of the start and end conditions.
A linear solvent gradient is calculated between two different solvent compositions at two different times. The percentage proportion of a solvent can either increase or decrease.

2 Flow Rate (ml/min). This function defines the flow rate (0.0 - 30.0 ml/min) at a given time. It is used either to program the flow rate independently of the preset value at time 0.0 or to change it at any time during the course of an analysis. The flow rate should not be altered during the course of a solvent gradient.
3 Max Press (bar). This function is used to define the pressure (0 - 490 bar) above which the pump automatically switches off.

4 Min Press (bar). This function is used to define the pressure (0 - 490 bar) below which the pump automatically switches off.

5 Oven Temp. This function is used to specify the temperature (-20 - 100^{o}C) of the column oven if installed.

6 Wavelength (nm). This function is used to select the required wavelength (190 - 600 nm) of a UV detector (spectrum scan cp. 5.1.3).

7 Attenuate (2^). This function is used to modify the attenuation (1 - 14) of a UV detector.

Attention! The attenuation only affects the Remote Control output of the detector.

8 Autozero. This function can be used to zero the detector signal from the UV detector.

Time-programmed changes with the last three functions can cause a major change in the detector signal which may negatively effect subsequent data handling (integration). Integration should be inhibited during changes of these 3 functions, this can be done with the Integrate Inhibit function in the data handling file.

9 Event. This function is used to switch ON or OFF the 4 available external events of the LC 21 pump from Bruker which may in turn be used to activate external devices such as switching valves, fraction collectors etc.
Having selected this function via 9, enter against Value the number of the external event required (1 - 4) followed by a space and the desired status (OFF=0 or off, ON=1 or on). The table above always contains the number against Value followed by ON or OFF.

10 Stop. This function allows you to stop the pump when injecting manually or semi-automatically. For example you can program Stop to a time which will never be reached when conducting a series of injections as the procedure is always being restarted at 0 by Start. However, once the injection series has finished this time will be reached in the LC-procedure and the pump will stop pumping solvent.

It is not "a must" to program the Stop function. The pump can also be stopped manually with the function key Stop Pump once a chromatogram has been recorded.

11 Emission (nm). This function specifies the emission wavelength of a fluorescence detector. The excitation wavelength is defined using function 6.

After clicking LC Procedure in the submenu of the Chrom.Files the "Open LC Procedure File" box appears for selecting of an existing file or entering of a new name (s. 4.1.1). After selection of a file name the file appears in a window in which the programmable functions are on the left, a time table on the right and at the bottom right an entry line. The cursor blinks in the Time box. Enter the desired time and key TAB to reach the box under Function. Enter the number of the programmable function, then jump with TAB to the Value box. You can also select a function by clicking it in the list at the left. Enter the composition of solvents in percent for each component separated by a space.
With Insert the selection is inserted into the table above. If more than 11 lines are entered the scroll bar appears to the right of the table.
If a line of the table is to be modified or deleted it is first clicked with the mouse. Then the line appears in the entry bar at the bottom where it can be deleted or edited and inserted back into the table.
The table can contain a maximum of 75 lines.

The file can be stored with ***File*** and one of its appropriate submenu commands.
A new file can be selected with ***File*** and ***New*** or the button  or *Open*...

Customize Function Names allows you to change the descriptions of the programmable functions (1 – 11) and to save these in CHRST32.INI in the section [Edit Files].

Atention! The new descriptions appear in all LC-Procedure files.

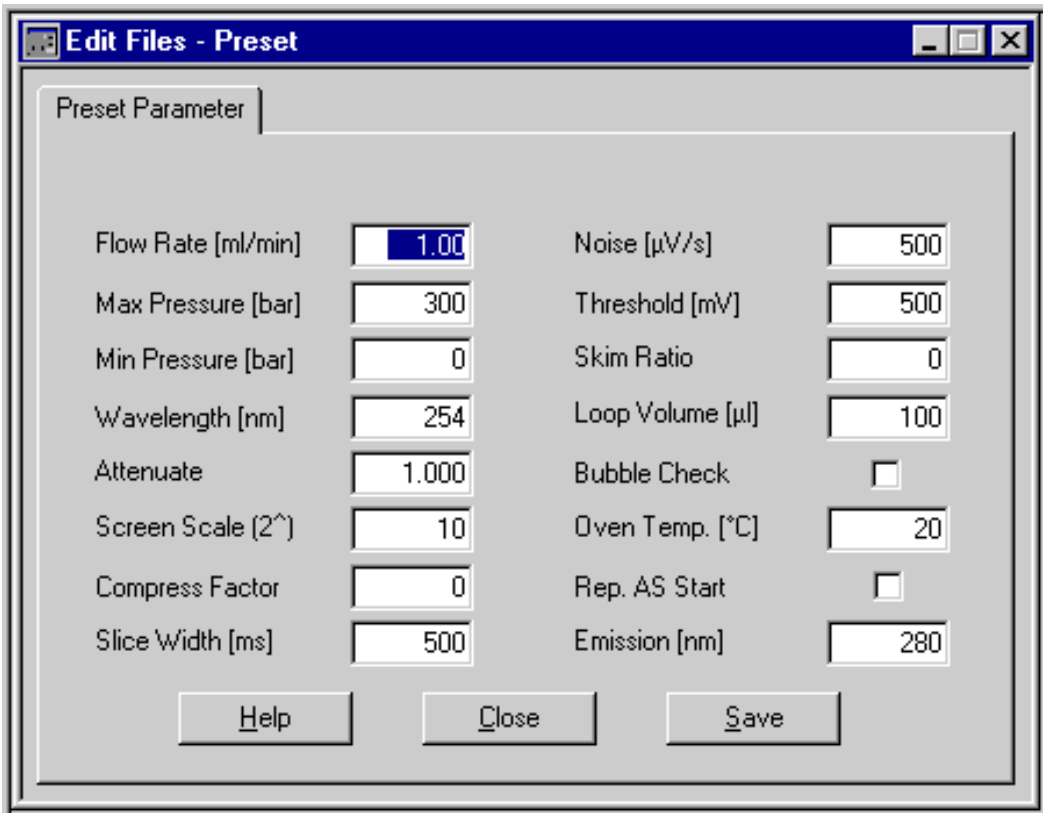
The original descriptions reappear on deleting the entries in CHRST32.INI in [Edit Files].

With ***Close*** or the button  the window is closed.

4.1.5 Edit Files - Preset

In the Preset File parameter values can be allocated which appear in the LC procedure and data handling files as default values. The threshold values of the individual parameters are given in the LC procedure and the data handling file. Only one preset file exists.

After clicking into ***Chrom.Files*** and ***Preset*** or using the button  the Preset Table appears on the screen.



The parameters of the Preset Table have the following meaning:

Flow Rate [ml/min]. Flow rate

Max Pressure [bar]. Upper pressure limit

Min Pressure [bar]. Lower pressure limit

Wavelength [nm]. Wavelength of the UV detector or excitation wavelength of the fluorescence detector, respectively.

Attenuate. Attenuation of the UV detector

Screen Scale (2^). Attenuation or amplification for the display of the detector signal on the screen

Compress Factor. Compressibility factor

Slice width (ms) Defines the period after which a new data point is recorded.

Noise [µV/s]. Maximum increase of the detector signal which is not recognised as the start or end of a peak

Threshold [mV]. Height over the current baseline above which detector signals of zero slope are not evaluated as new baseline

Skim Ratio. Ratio of the heights of two adjacent peaks to that of their common valley (4.1.2.2).

Syringe vol [µl] defines the syringe volume of the autosampler LC 51 to 50 or 250 µl, respectively, according to the syringe used in the autosampler.

Bubble check defines whether the LC 51 autosampler has to perform an air bubble check after each injection or not.

Oven temp. [^{0}C] regulates the temperature of the column oven.

Rep. AS. Start defines whether a run has to be continued or not after an error message during an autosampler run.

Emission [nm]. Emission wavelength of the fluorescence detector

With ***File*** and ***Save*** the altered parameter values are set as default values.

With ***Close Preset*** or the button  the preset table is closed and the Edit Files window reappears on the screen.
Before the changes are saved the question appears "Save current changes?", which is answered with yes.
Then the preset table is written to the CHRST32.INI file into the section [Preset].

4.2 Analysis - Chromatogram

Data Acquisition - Pump Control

In the application **Analysis - Chromatogram** the detector signal is displayed on the screen. **Analysis - Chromatogram** allows you to record a chromatogram and to control HPLC devices. The devices to be controlled are connected to the computer via the RS232 port.

If menu points and commands in this application appear in light grey print they cannot be accessed. This happens when the necessary preceding steps of an application have not yet been carried out.

In order to record a chromatogram (further details see section 5.1.1) you must first, in the **Edit Files** window, have created and stored a method file and a data handling file and if an HPLC pump is to be controlled an LC procedure file. The method file should contain on the first page the entry Mode = HPLC (or GC) and the name of a data handling file for the first data recording channel.

The further entries to be made in the method file are explained in section 4.1.1. The parameters defined for the data handling file are described in full in section 4.1.2.

Attention! The files required for carrying out an analysis - method file, data handling file, and the LC procedure file when using a controlled pump - must be stored in the same directory. This is also the directory where recorded chromatograms (slice files .SLI and report files .RPT) are stored.

After clicking the application ***Analysis - Chromatogram*** a dialog box appears for **selection** of the data acquisition channel and pump control (see next section).

The menu bar contains the menu points

Method... Operation HPLC Display Options Window Help

in which only the menus *Method...*, *Options*, *Window* and *Help* can be selected. The sub-menus of the points not yet to be used appear in grey letters.

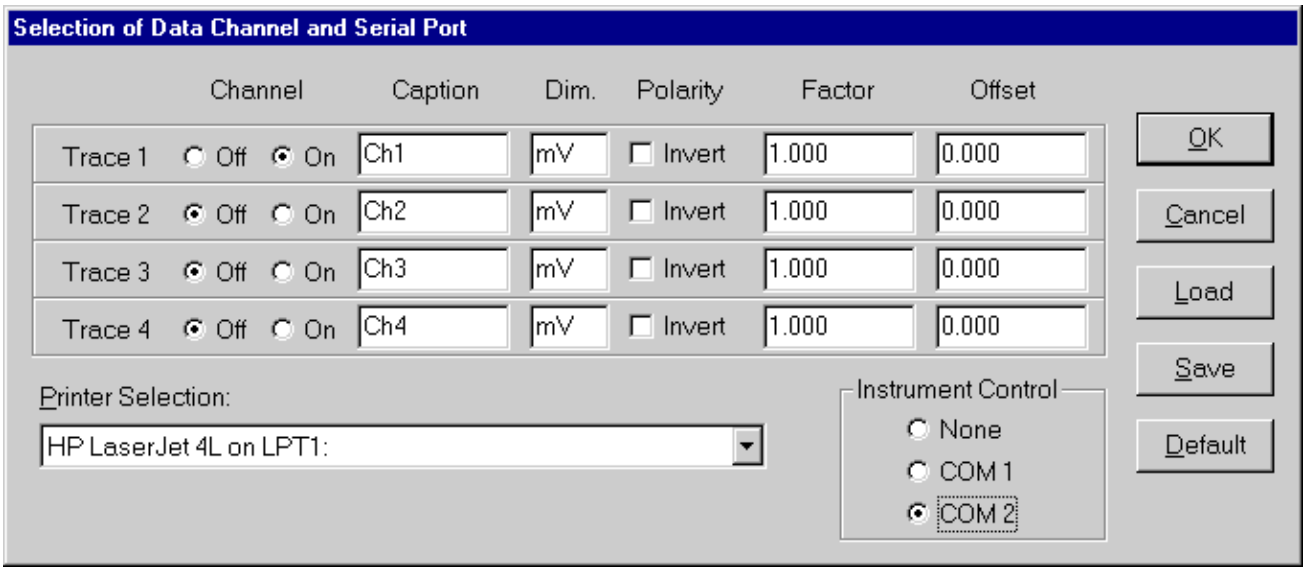
In the **Analysis** application the **Toolbar** shows besides the buttons to the right for the ChromStar main applications a number of buttons for quick and easy operation. These are at the beginning of the work in Analysis mainly grey. In the following sections the menu points (and buttons) are described in the sequence in which they are normally used when carrying out an analysis.

If independent chromatograms should be recorded, the application **Chromatogram** can be opened once more via ***Window*** and ***New***. After entering the data channel and printer (if necessary) to be used in the second Analysis window in the **Selection**-box a method can be started via ***Method...*** and entering a file name. Up to four independent **Analysis –Chromatogram** applications can be opened.

Using multi-channel data acquisition in one **Analysis-Chromatogram** window the data handling files entered in the method file starting with DH(1), DH(2) etc. are used for data acquisition and integration. All chromatograms are recorded with the slice width and the run time entered in DH(1).

4.2.1 Analysis - Chromatogram - Options - Select Channels...

With ***Options, Select Channels...*** the data recording channel to be used (*Trace 1* to *Trace 4*) is defined whereby a maximum of 2 or 4 channels (depending on the number of A/D converter boards installed) can be selected to operate in parallel. The serial number of the A/D-converter board is stored in the chromatogram header.



For each data channel the name can be defined under *Caption*, and the dimension under *Dim*. The detecor signal can be multiplied by a factor (*Factor*) and an offset (*Offset*) can be added. If a detector signal is expected to have a negative polarity it can here be switched over by clicking into *Polarity* and *Invert*. These entries are saved in the CHRST32.INI file in the section [channeldef1] etc.

Also a connected LC device via RS232 (*COM 1* or *COM 2*) can be selected here.

The printers installed are shown in a list after clicking the downwards arrow in the *Printer Selection* box, now the printer to be used can be selected.

After clicking ***Options, Select...*** or when first opening the Analysis window the desired options are marked with the mouse cursor.

The selections can be stored in a file (.RSE) with *Save* and recalled later with *Load*.

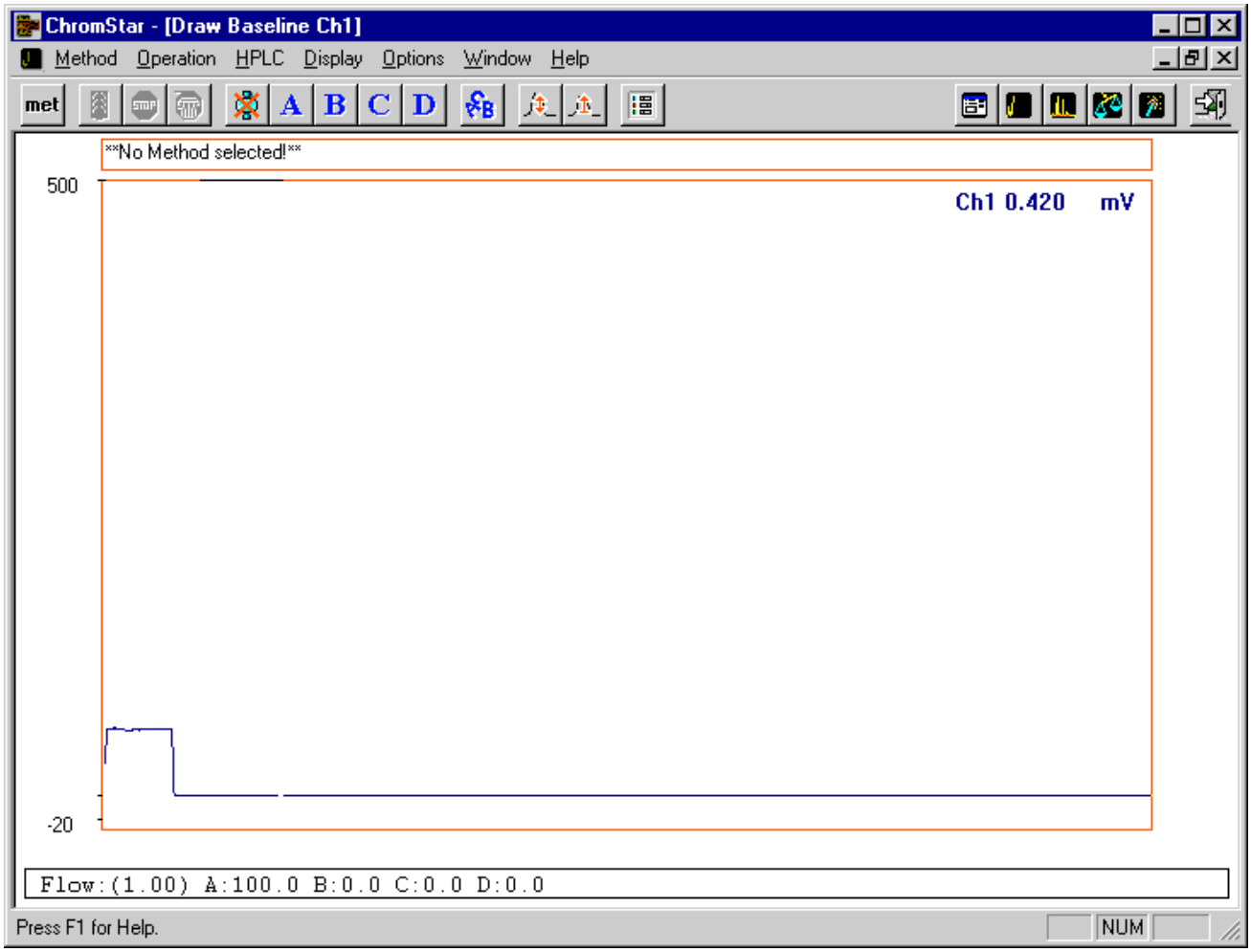
A selection can be saved as DEFAULT.RSE by clicking into *Default.* This selection is automatically carried and the 'Draw Baseline'-window is shown when Analysis is used later on. The *Default* key is only visible, when in Chrst32.INI in the section [Configuration] the entry is DefaultButton=1. The entry DefaultButton=0 or no entry causes the button to be invisible, the default.rse file will be deleted.

Quit with OK. Prior to doing this the HPLC equipment should be switched on.

Now a window with the title **Draw Baseline Channel 1** appears and the baseline of the detector attached to this channel is displayed on the screen. The digital display of the mV signal appears in the right top corner of the **Draw Baseline** window behind *Ch1*, (*Ch2* etc).

When HPLC devices are controlled details of their status are displayed in the line below the draw baseline window.

In this display the number of the vial analysed in the autosampler is shown behind *Vial*, the number of the injection from this vial behind *No*, and the rate at which the eluent is being pumped behind *Flow*. The percentages of the solvents are shown against A, B, C and D. *Wave* indicates the wavelength of the detector in nm and *elt* the time elapsed since the start of the chromatogram.



After ending the selection the menu points ***Method***, ***HPLC*** (only accessible when controlling a pump), ***Display***, ***Options***, ***Window*** and ***Help*** can be accessed.

When acquiring data of more independent channels, you must open more than one (2, 3 or 4) Analysis application window.

4.2.2 Analysis - Chromatogram - HPLC

The menu point ***HPLC*** is only accessible when a pump is controlled via RS 232 and an appropriate interface. The sub-menu points or the buttons, respectively, are used for the pump control.

Stop Pump stops the pump immediately. No dialogue box appears. The pump can be stopped even quicker by striking the function key F9.

Flow Rate determines the flow rate in ml/min and can be varied between 0.0 and 30.0. The flow rate can also be defined with the F10 function key.

After clicking of ***Solv A***, ***B, C*** or ***D*** the pump starts pumping the solvent chosen. Also the function keys or the buttons can be used for choosing the solvent without clicking the *Pump* menu.

F5 = Solv A

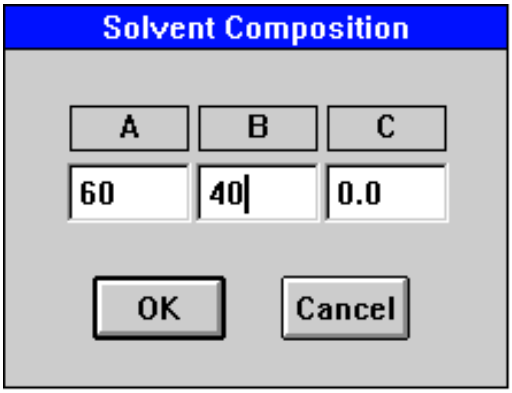
F6 = Solv B

F7 = Solv C

F8 = Solv D

Click the submenu point ***Composition*** if an isocratic mixture from two different bottles is to be pumped. The percentage amount of each solvent is entered in an entry box, the portion of D is automatically calculated.

After clicking Ok the pump starts pumping the defined solvent composition. In the status line below the **Draw Baseline** window the specified solvent composition is displayed behind A, B, C and D.



An LC procedure (4.1.4) must be written if a solvent gradient or a stepwise change of flow rate is to be pumped. After this a method must be selected (4.2.3) and started which contains this LC-procedure.

The programming and operating of a slow solvent change is described in section 5.1.2.

The wavelength, autozero and the attenuation factor of the UV detector or the excitation and emission wavelength of a fluorescence detector, respectively, are programmed with ***Wavelength***, ***Emission***, ***Autozero*** and ***Attenuation***. With ***Oven*** the temperature of the column oven is selected.

Necessary for this are a UV-detector and column oven which can be controlled. These instruments have to be equipped with the appropriate interface.

Pump status

The status line at the bottom of the Analysis window shows the information about the pump control.

Vial: Vial number (when an autosampler is in use)

No: Sample number or injection number, respectively

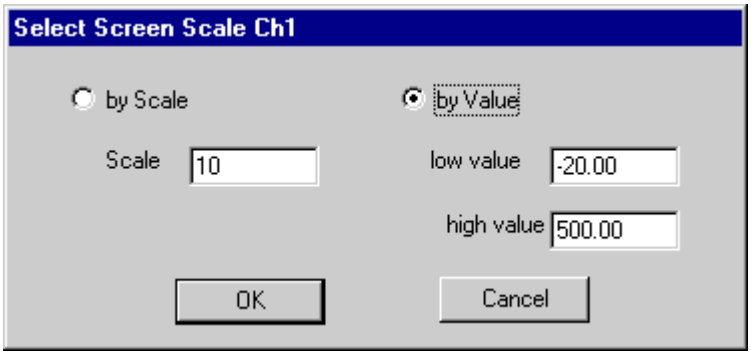
Flow: Flow Rate in ml/min

A:B:C:D: Eluent composition in %

4.2.3 Analysis - Chromatogram - Display

The menu point ***Display*** allows you to change various screen display parameters.

The vertical axis of the screen display of the detector signal can be changed by defining an other attenuation factor (***Screen Scale***) or an other mV value. These changes are entered in the Select Screen Scale box, which is accessed either with the F11 key or via the menu option ***Display*** and the submenu ***Screen Scale.***



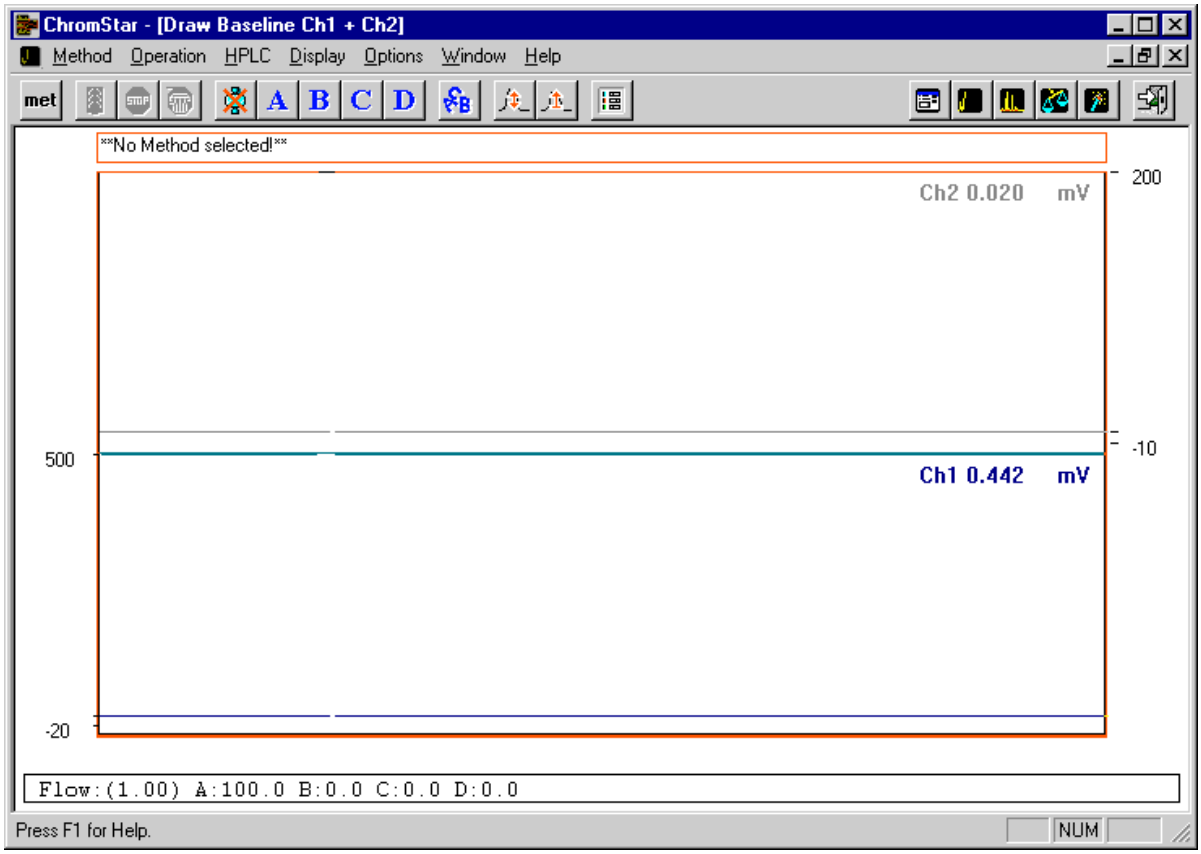
After clicking *By Scale* an attenuation factor can be entered in the box after *Scale*. 10 corresponds to a range of 0 - 313 mV, 9 corresponds to 0 - 156 mV, etc. After clicking *By Value* a fixed mV-range for the representation on the screen can be defined by entering the relevant values against low value and high value. These entries are saved in CHRST32.INI and are used when opening the application again.

When using more than one data recording channel the box for channel 1 is the first to appear. Enter the desired value and then click OK or strike the enter key and the box for channel 2 appears in which you can enter the scale of the vertical axis for the representation of the second channel etc. Cancel aborts the operation.

Clicking the command ***Normalize*** (or function key F12) during a chromatogram recording sets the lowest data point at the lower border of the display, provided that the screen scale is defined by Scale. In this way the chromatogram is still visible on the screen even if the baseline has drifted away.

Fullsize re-establishes the chromatogram in full size e.g. after using the ***Unlimited Runtime*** option (cp. sect. 4.2.5). While recording a chromatogram an enlargement can be made by using the mouse. With *Fullsize* all recorded data points are represented on the screen again. This can also achieved by using the right mouse key in the data acquisition window.

When 2 or more channels in one analysis are being used for recording data the representation of the chromatograms on the screen can be done in two ways. The submenu point ***Stacked*** divides the screen so that the lower half displays the channel 1 chromatogram in yellow and the upper that of channel 2 in green. If 4 channels are being used channel three is displayed in blue, channel 4 in cyan-blue.



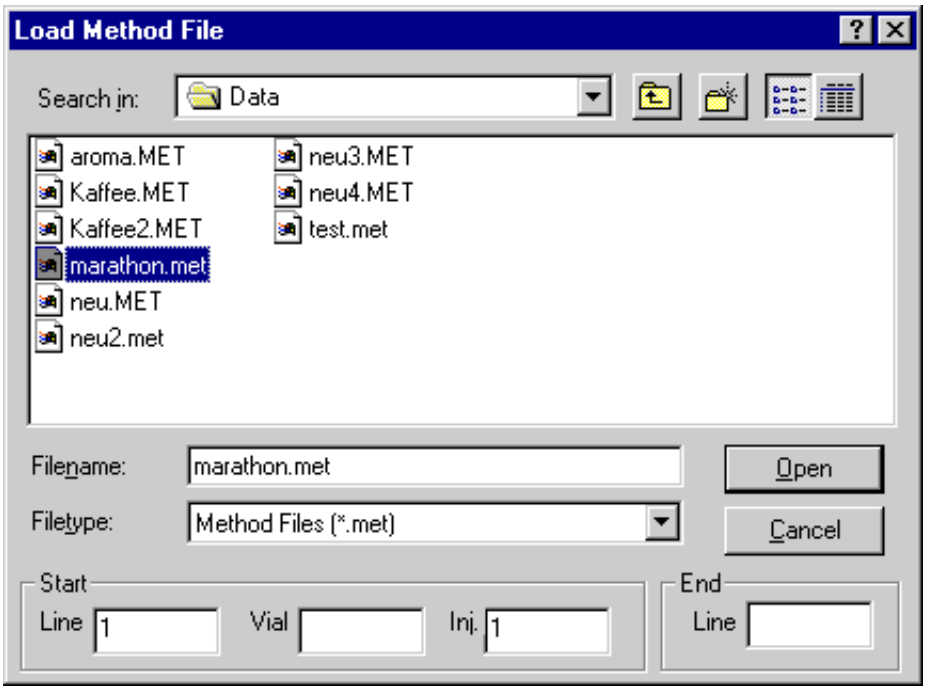
Selecting the submenu point ***Overlayed*** displays the chromatograms in a superimposed graphic with a common x and y axis. The channel 1 chromatogram is yellow, that of channel 2 is green.

Show Comparison Chromatogram is used to show or hide the comparison chromatogram, which had be loaded under ***Method.*** The *Slice Width* parameter must be the same in *Edit Files, Preset* and in the chromatograms.

Show Gradient. When a method with a gradient in the LC procedure file is loaded, this menu procedure can be used to show or hide the gradient in the data acquisition window.

4.2.4 Analysis - Chromatogram - Method...

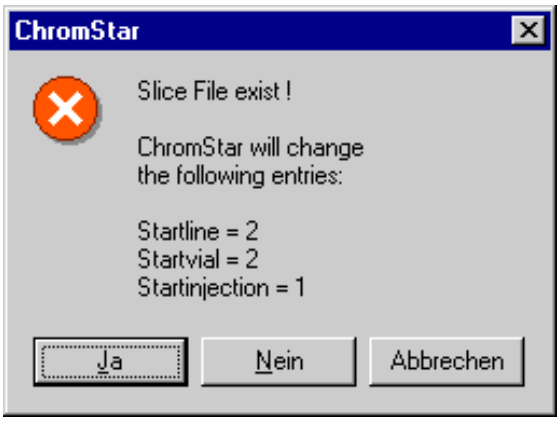
After clicking into ***Method..., Load*** you must first enter the name of the desired method in the Select Method File window.



You can select the drive and the directory in the box after *Search in:*. A method file is selected by clicking a name in the list or by entering the name against Filename. If an autosampler is being used the start point for a series of samples to be run can be defined by entering against *Line* the line number of the autosampler table, against *Vial* the vial number and against *Inj*. the injection number. If no autosampler is being used the injection number specified in the Sample Table of the Method File can be entered against *Inj*. to define the start point of the analysis. A check is made whether the chromatogram already exists.

If this is the case one of the following messages appears:

An existing chromatogram can not be overwritten. To continue this method when using an autosampler a higher start line must be entered or the Data-File name must be changed in the Method-File or the existing chromatogram must be deleted.



If no autosampler is used a number has to be entered, before the start-injection, under which no chromatogram is stored yet.

If more than one injection out of the same vial is made by editing the vial in different lines of the autosampler table, an error message under *Error...* appears when the second chromatogram is to be saved since the chromatogram with the same name already exists. The already existing chromatogram is moved into the TEMP00.. directory (cp. p. 5.1-13).

If an autosampler method was completely carried out and is called again, the message appears: *Slice File exists. Overwrite all chromatograms? Yes/No*. After answering No the method can be cancelled, the chromatogram name in the method can be changed. After answering Yes the message appears: *All stored chromatograms of this method will be deleted. Continue?* With OK all chromatograms will be deleted and the method can be started.

After leaving the *Load Method File* window with OK the method name is displayed in the line above the signal area in the **Draw Baseline** window. When using a controlled HPLC pump the initial solvent composition of the LC-procedure used in the method will be pumped through the whole system. On leaving the window with Cancel the Message "No Method selected“ appears in the information line.

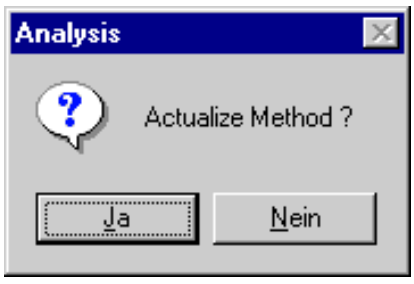
As soon as pressure and detector signal have stabilised and the column is conditioned, the system is ready for recording a chromatogram.

The method can be started from the menu point ***Operation, Start*** (cp. next section), the injection has to be carried out simultaneously.

Injection and start of the chromatogram can be synchronised via start input on the A/D-converter board (cp. p. 2-2) by a signal from the autosampler or the injector, respectively.

When using a controlled autosampler, the injection of the autosampler is initiated by the control interface.

Method..., Actualize. If changes are made in the method file in **Edit Files** after starting the method and recording a chromatogram, these changes can become active by using ***Actualize*** when the following injections are carried out.



In the method file, *Run Table* the following changes can be made: *Notes*, *Normalization* and *Report*, after ***Save*** and ***Actualize*** the changes are active in the same run. A change of *Use Linenumber* is only active in the next run.

Attention! A change of *Data File(S)* (=name of the chromatograms) is not possible.

In the *Autosampler Table* **after** the line in process, a line can be inserted, an existing line can be changed or a line can be appended. After ***Save*** and ***Actualize*** the changed method will be carried out.

Attention! The line of the autosampler table in process may not be changed.

In the *Sample Table* the parameters *Sample Identifier, Sample Info, Factor, Weight* and *Int. Std* can be changed. After ***Save*** *a*nd ***Actualize*** the changes are active for the next injection.

Changes in the *Documention Table* are active in the same run after ***Save*** and ***Actualize***.

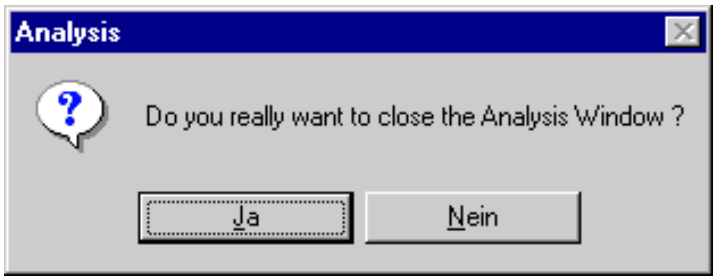
When the last line of the autosampler is being processed, no changes can be made. On doing so, the message appears: *Can't actualize*.

If modifications are made in the data handling file and saved, these are active from the next injection without Actualize. Changes in the calculation file are active in the same run without Actualize. Changes in the LC procedure file are active from the next line of the autosampler without Actualize.

After changes in the various files the method can be stopped, activated again and be restarted by entering the line number, the vial and the injection number,

Comparison Chromatogram. An existing chromatogram can be loaded for comparison reasons. The parameter *Slice Width* must be the same in *Edit Files, Preset* and in the chromatogram. The chromatogram is shown in the Analysis window and the menu point ***Show Comparison Chromatogram*** under ***Displayis*** is marked.

Method, Exit, the button or the x-Box close the data acquisition window. The question appears:



The question is answering as desired.

4.2.5 Analysis - Chromatogram - Operation

The ***Operation*** submenu contains the following commands to carry out a chromatographic run:

Start Start the method (and therefore the recording of the data) (also using funktion key F1)

Stop End of data-acquisition (F2)

Stop Int. Stop integration
if no autosampler is used also stop recording

Abort Abort recording (F3)

Continue Continue the method, only operational if an autosampler is used

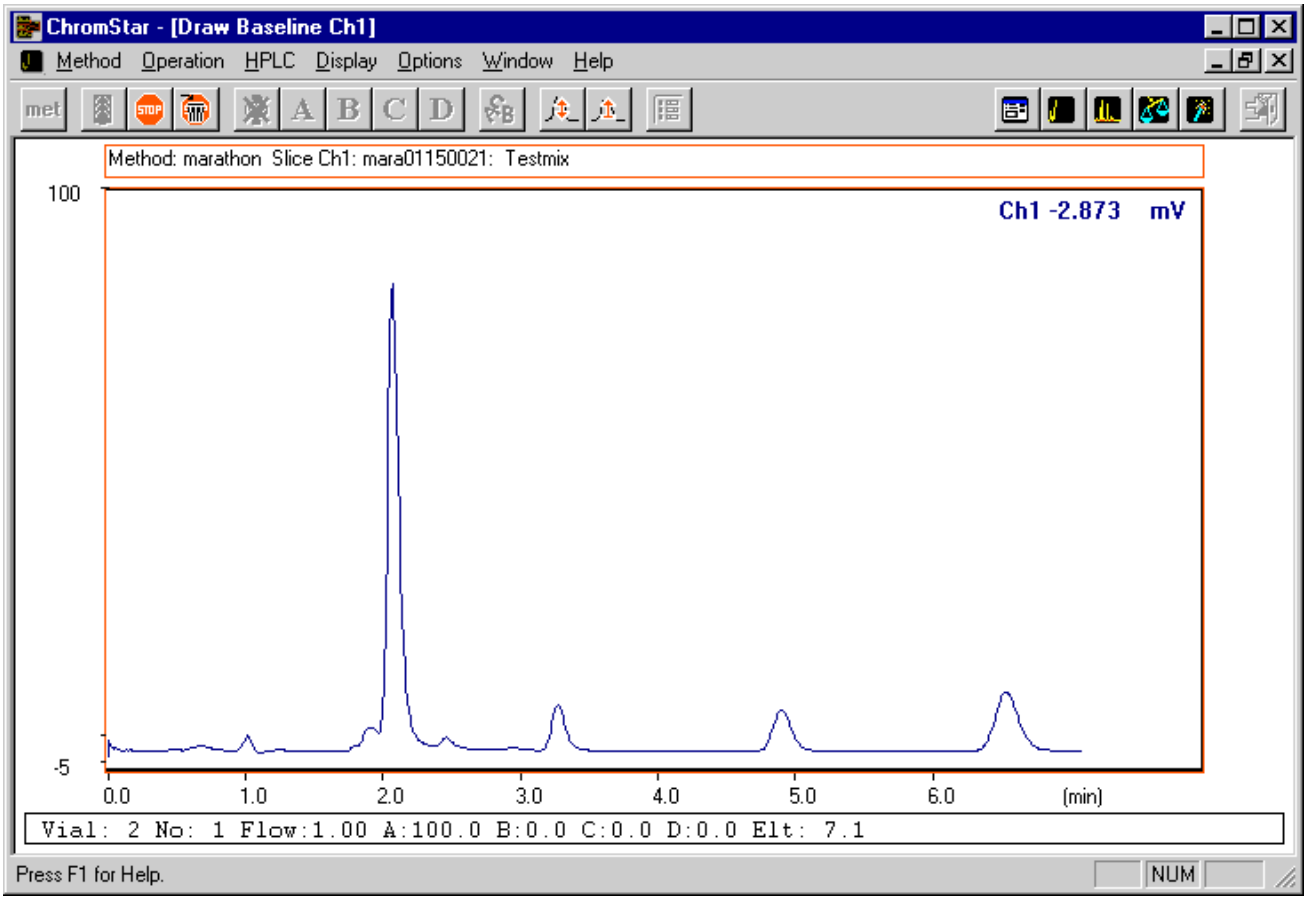
Unlimited Runtime allows the runtime of a chromatogram to be increased.

These commands are activated by clicking with the mouse. Before the method is started only the *Start* and *Unlimited Runtime* function can be accessed. After the method has been started the functions *Stop*, *Stop Int.*, *Abort* and *Continue* (only when using the autosampler) can be accessed.

The ***Start*** command is given at the same time as the injection. This can for instance be done automatically, initiated by switching of the injector (with aid of the LC 427 Event-Box). When an autosampler is used an automatic start is also performed at each injection.

There is no need for the analysis window to be activated in the case of an automatic start.

The processing of the time table of an LC Procedure with a solvent gradient begins at the start time.



The window now carries the title **Data Acquisition Channel 1**. The names of the Method and the currently recorded chromatogram are displayed above the data-field. The time elapsed since chromatogram start is displayed against Elt: in the line below the data-field.

Stop initiates the premature evaluation of data recorded up to then, and generates a print-out (depending on the report print template, e.g. **6**). The chromatogram and the Report-File are stored. If an LC-procedure is being used with entries in the time table, processing is terminated with *Stop* and the current eluent composition will continue to be pumped.

Attention! A chromatogram must not be terminated with Stop. Recording will normally continue for the time specified in Run Time of the data handling file. In this case the LC-procedure is also normally processed until the end.

Stop Int. causes the integration to stop at the time this command is executed. The chromatogram recorded up to this moment is stored and printed out. If an LC procedure file is used with a time table, it will normally be processed until the end.

Abort stops the data acquisition. No raw data are stored; no evaluation takes place and no print-out is made. The processing of the time table in the LC-procedure is aborted and the current eluent composition will continue to be pumped.

The commands *Stop, Stop Int.* and *Abort* need to be confirmed after selection with OK in a dialog box before they are executed.

Continue starts the next injection when using an autosampler. Data recorded up to then are evaluated and a print-out is made (not yet operational).

Unlimited Runtime After clicking into this sub-menu procedure the chromatogram does not end automatically at the elapsed run time, it can be stopped manually at any later time. A corresponding message appears in the title line. When using an autosampler the chromatogram must be stopped using *Continue* . The autosampler run is then continued by carrying out the next injection. After the data-handling run time is elapsed a scroll bar appears at the lower frame which can be used to show the last recorded data. This can also be achieved by using *Control, Fullsize.*

The function keys are used without calling the Operation menu point from which these functions can also be activated.
If more **Analysis** windows are open, the function keys operate only on the active window which has a red frame.

If you try to close an Analysis window or to quit ChromStar or WINDOWS with the control menu box or Exit while a chromatogram is running, a box is displayed with the warning.

4.2.6 Analysis - Chromatogram - Options

Options offers various options in its sub-menu.

Select Channels... opens the window for selection of data channels and serial ports (cp. section 4.2.1).

Error. The function of this menu point is to make a reconstruction if an error occurs. Errors occurring during an automated analysis are registered and stored in a file (.ERR). The field "Error View" can be accessed through the menu point ***Error....***

A list with errors that may have occurred can now be printed.

Using *Open* other error files can be opened. An error file can be deleted by *Delete*. *Cancel* closes the *Error View* window.

When another method is opened and started the error window is automatically emptied.

With ***Automatic Noise*** an automatic measurement of the noise value can be performed. It is necessary for this evaluation to have acquired enough data points in the computer memory. If this is not yet the case the message appears

"Not enough data points acquired".

After a successful evaluation a dialog box appears with the determined value. The noise value is determined so that no peak recognition occurs during this period. Clicking Ok enters this value to the preset file and in all data handling files were it is entered as preset value. This value is used in the evaluations if no defined Noise value is entered in a data handling file (integration page) at time 0. Clicking *Cancel* prevents this value to be entered into the parameters files.

4.4. Reprocess - Integration

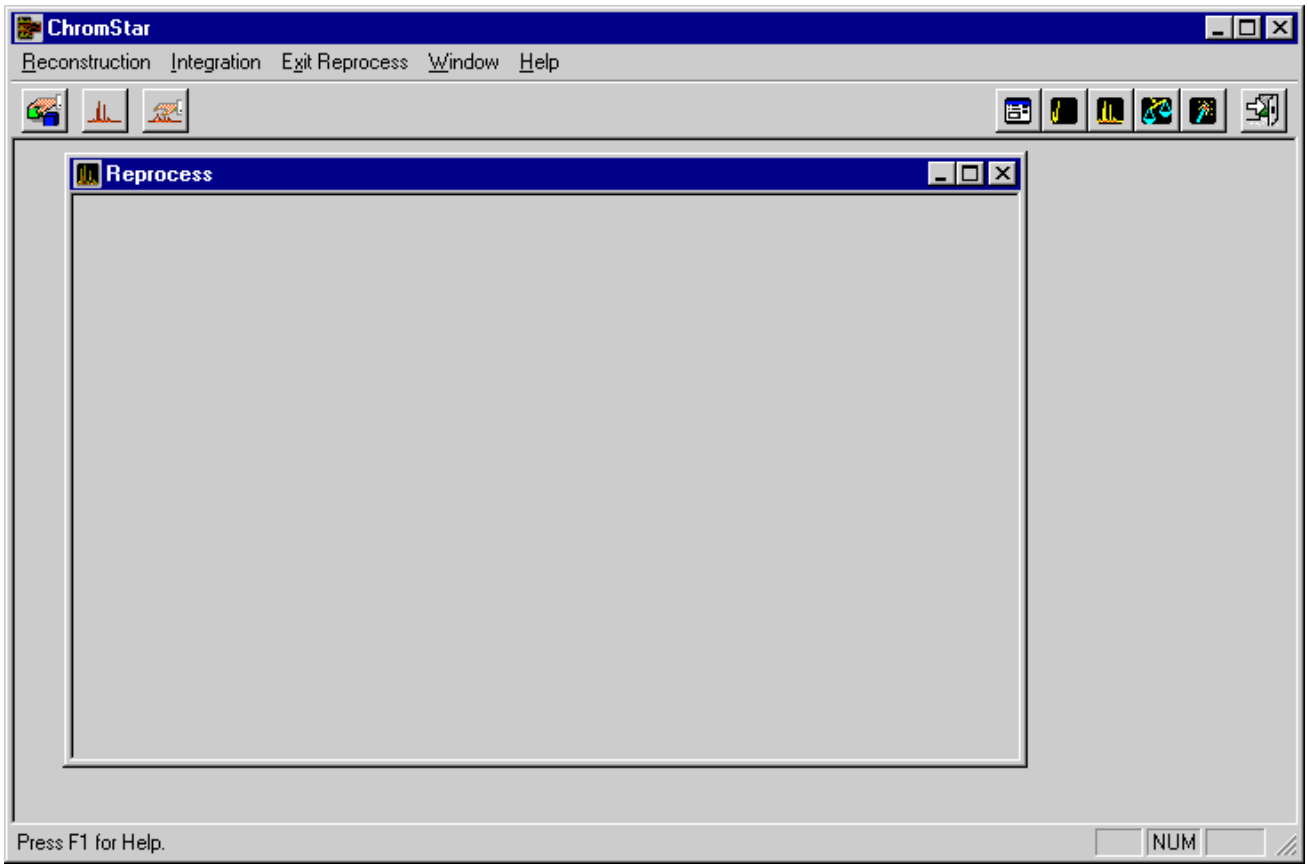
Reprocessing of Chromatographic Data

The application **Reprocess** offers the facility of reprocessing chromatographic data. Individual chromatograms can be reconstructed, in full size or in detail; chromatograms can be reintegrated, either automatically or manually. The **Reprocess - Calculations** window offers still more possibilities to handle chromatographic data. These are described in section 4.5.

Selecting **Reprocess** in the menu bar of the ChromStar system window with the mouse or by using the button opens a new applications window with the title bar Reprocess. The individual points of the menu bar

Reconstruction Integration Exit Reprocess Window Help

can again be accessed with the mouse arrow and selected by clicking the left button of the mouse which briefly underscores the menu point selected in black with the exception of *Integration* where a submenu appears.

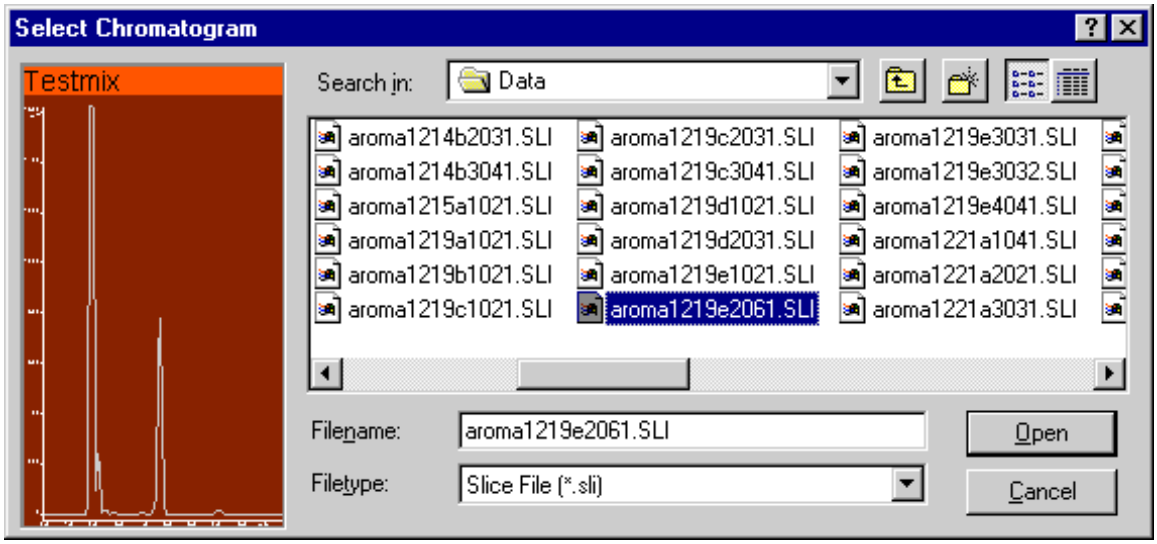


An icon of the application can be created by clicking the third box from the top at the right (icon box).

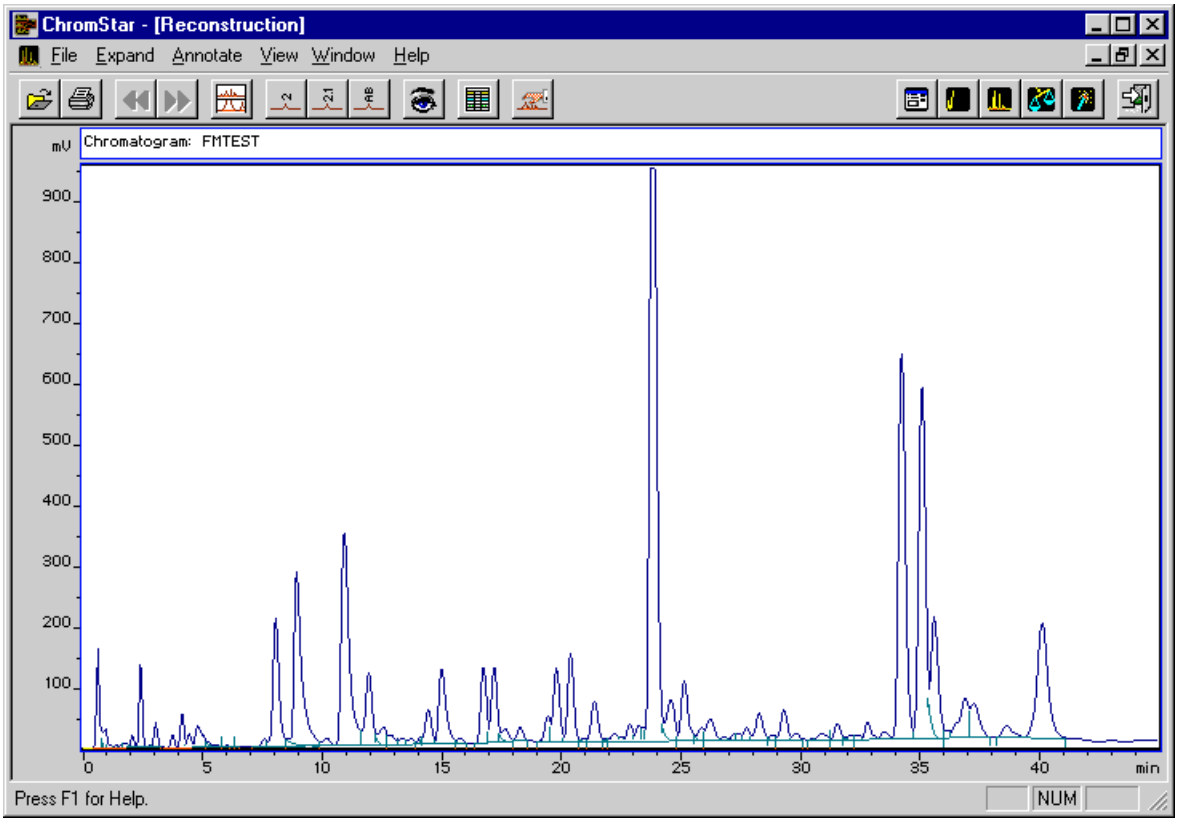
The individual menu points are described in the following sections.

4.4.1 Reprocess - Reconstruction

Clicking the menu point ***Reconstruction*** opens the dialog box Select Chromatogram for selection of a chromatogram to be displayed on the screen.



The drive and directory can be chosen in the top. The chromatograms are listed in the middle. After clicking on one of the names a preview of the chromatogram and the *Sample identifier* - if any was entered when recording the chromatogram - appear in the box to the left. If another name is clicked the preview changes accordingly thus offering immediately information about the chromatogram and the *Sample identifier*.



After selection of its name the chromatogram appears in the full area of the application window with the integration carried out last (which is saved in the corresponding .RPT file).

The title bar carries the application name (**Reconstruction**), the menu bar contains the following menu points:

File Expand Annotate View Window Help

The buttons in the Toolbar to the left allow quick access to the main operations in this application. They are described together with their corresponding menu points. In the line above the chromatogram the message appears: "Select rectangle to zoom". An enlargement can be made by setting up a rectangle with the mouse (cp. p. 4.4-5). By clicking with **the right mouse key** in the chromatogram the original size is restored.

The menu point ***File*** permits the following file operations:

Open... returns the user to file selection in order to display another chromatogram.

Open Previous selects the chromatogram, in a series of chromatograms, with the next lower number at the end of the chromatogram name.

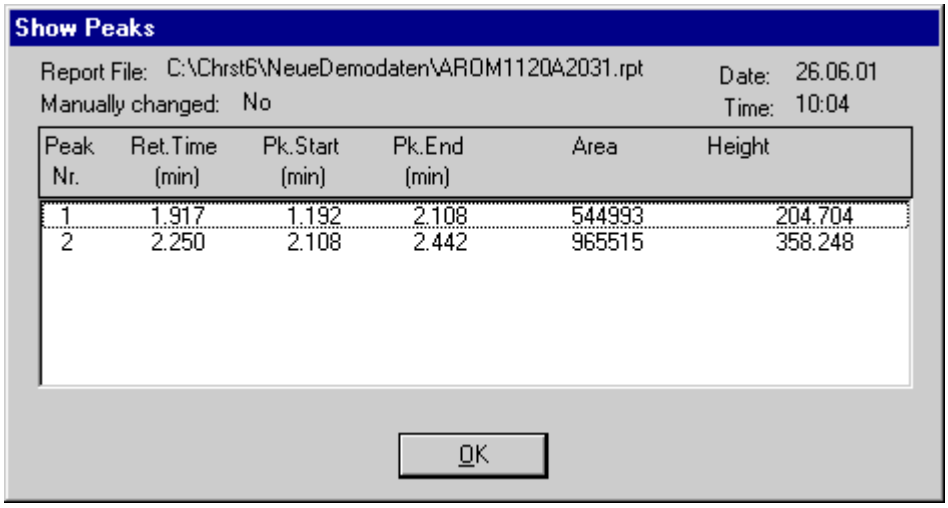
Open Next opens the chromatogram with the next higher name.

Both menu points are light grey when no chromatogram with a higher or lower number exists.

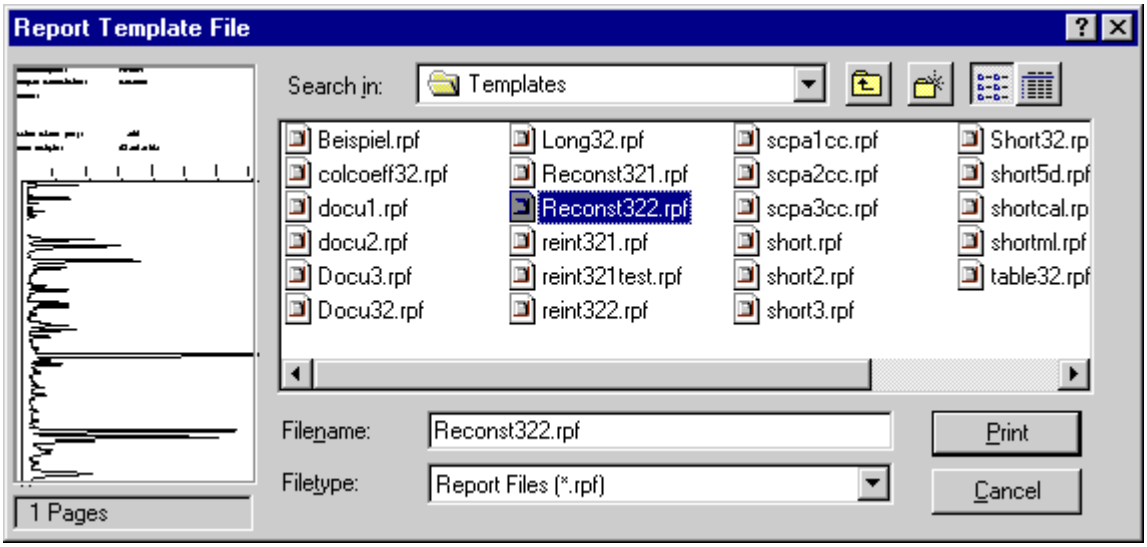
Comparison Chromatogram loads a comparison chromatogram, the slice width must be the same as in the reconstructed chromatogram.

With ***File Information*** the following entries concerning the chromatogram are displayed: Author, Sample Identifier (entered in the sample table of the method file, which is used during recording of the chromatogram), date of recording, changes, number of data points, run time, lowest data point and highest data point.

Show Peaks from File... opens a file select box , where a report file can be chosen, the preview on the chromatogram is shown to the right. After clicking OK the report list appears on the screen.



Print permits the printing of a chromatogram. After clicking *Print* a selection box appears in which you can select the format of the chromatogram print-out.



The directory containing report template files can be chosen on top of the box. The report templates appear in the list to the right. The print preview appears in the box to the left. On deselecting *Watch* the preview disappears.

With ***Printer Setup*** printer settings like resolution, paper size, portrait or landscape orientation can be changed.

Copy with ist sub-menus ***Chromatogram*** and ***Report*** transfers the chromatogram or the results list to the WINDOWS Clipboard where it is stored temporarily. Accessing ***Copy*** a second time will overwrite the previous contents of the clipboard. The contents of the clipboard can for instance be viewed with the WINDOWS application Editor. This can be done by opening the editor and transferring the contents with the *Paste* command from the *Edit* submenu. With *File* and *Save* you can now create a text document into which the required information from ChromStar is inserted via the clipboard.

Save as... allows a chromatogram to be saved under a new name. The corresponding report file (.RPT) is created simultaneously.

Export... opens a file select box where a print report template can be chosen. The chromatogram can be saved as WMF-file in the representation of this template and can then be represented in an appropriate program for grafic display.

Reintegration displays the entry box Select Chromatogram and DH-File. After selecting the files it allows you to make a quick transition to the application Reintegration (cp. 4.4.2.1).

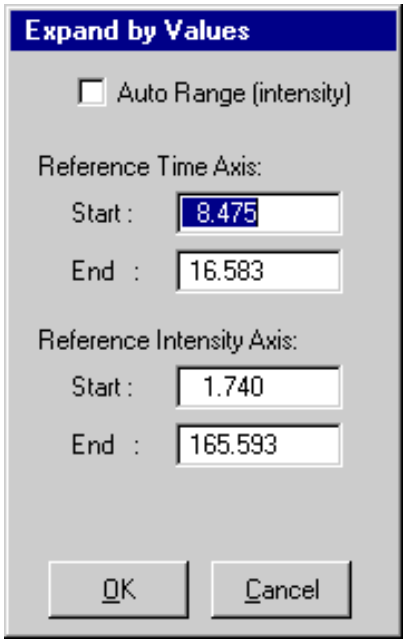
Manual Integration opens the application **Manual Integration** (s. 4.4.2.2).

Exit Reconstruction closes the **Reconstruction** window.

Expand allows a section to be selected from the entire chromatogram. After clicking ***Expand*** a submenu appears with the points ***Reference Window***, ***By Values...*** and ***Default***.

Reference Window allows a section to be selected by drawing a rectangle. With ***Auto range*** the selected section is automatically normalized to the highest peak.

An enlargement by setting up a rectangle is made as follows: The upper left hand corner is defined by moving the mouse arrow to the point required and clicking with the left button. The lower right hand corner is defined by moving the mouse which draws a rectangle, the position is confirmed with the left button. This causes the mouse arrow to return to the upper left hand corner which can now be corrected and confirmed by clicking the left mouse button. The rectangle is confirmed by clicking the right button of the mouse. The section of the chromatogram appears in the window. Clicking the chromatogram with the right button again restores the previous size.



The submenu point ***By Values...*** allows you to zoom out by entering the time and mV values in a dialog box. The initial values in this dialog box represent the current size of the chromatogram. These initial values are also adapted after utilising the ***Reference Windo****w* procedure.

Default (or clicking with the right mouse button) restores the original display of the entire chromatogram.

Annotate, Automatic allows the user to mark peaks in a chromatogram by numbers, by retention times, or by names.

When ***Peak Numbers*** is clicked, the chromatogram with its peaks numbered is displayed.

Clicking ***Retention Times*** annotates the retention times above the individual peaks.

When using a calculation file with entries in the peak table only the peaks of the peak table are labelled if:

Annotate = Results

is entered in the CHRST32.INI file in [Report].

If all peaks are to be annotated, the entry (or no entry) must be:

Annotate = All

To annotate ***Peak Names*** it is necessary to have defined them in the calculation file which is specified in the data handling file used in the reintegration. The peak names to be annotated are entered on page 2 (peak table) of the calculation file. The peaks are defined according to the corresponding peak windows.

The submenu point ***Manual*** allows you to write individual comments horizontally or vertically on the chromatogram in addition to the possibilities already described. After clicking *Manual* a box appears in which the direction - ***Horizontal*** or ***Vertical*** - is defined. Then with the right mouse button click the area of the chromatogram at which the text is to appear. The text is then entered in a text box with a maximum capacity of 30 characters in the horizontal and 20 in the vertical direction.

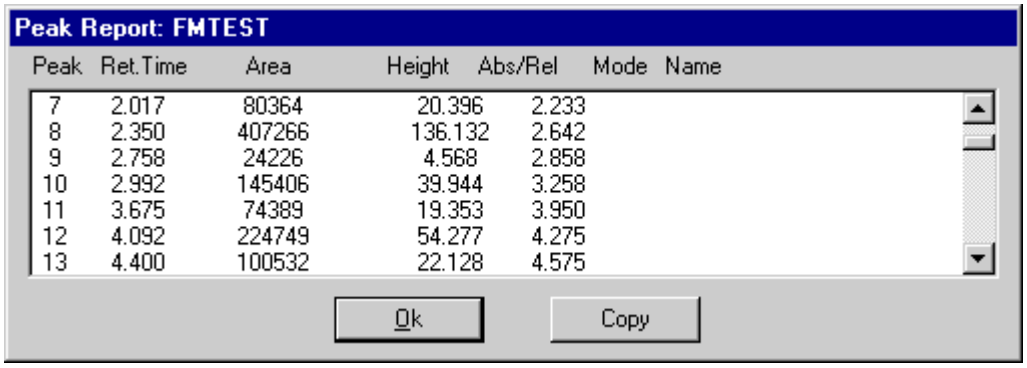
A previous annotation can be erased with ***Delete last annotate***. All manual annotations can be erased one after the other by clicking the menu points *Annotate, Manual* and *Delete last annotate*. The annotation appears only on the current screen presentation, on the print-out and on the presentation transferred by means of *Copy*.

The menu point ***No-Annotate*** deletes an existing annotation. This can be done easier by clicking the appropriate pressed button.

View with ist sub-menus ***Results, Show Comparison Chromatogram, Split, Show Values, Show Gradient*** and ***Audit Trail*** allow additional displays.

With ***Results*** the results list is displayed on the screen in the same. The table contains peak numbers, retention times, peak areas and peak heights, the result of the last calculation under Abs/Rel., and peak names. Manually integrated peaks are marked by an M under Mode. The scroll bar at the right of the table allows you to scroll through the list if it is longer than the screen page. The dialog box may be moved at choice over the screen by placing the mouse cursor into its blue title bar, pressing the left mouse button and dragging it.

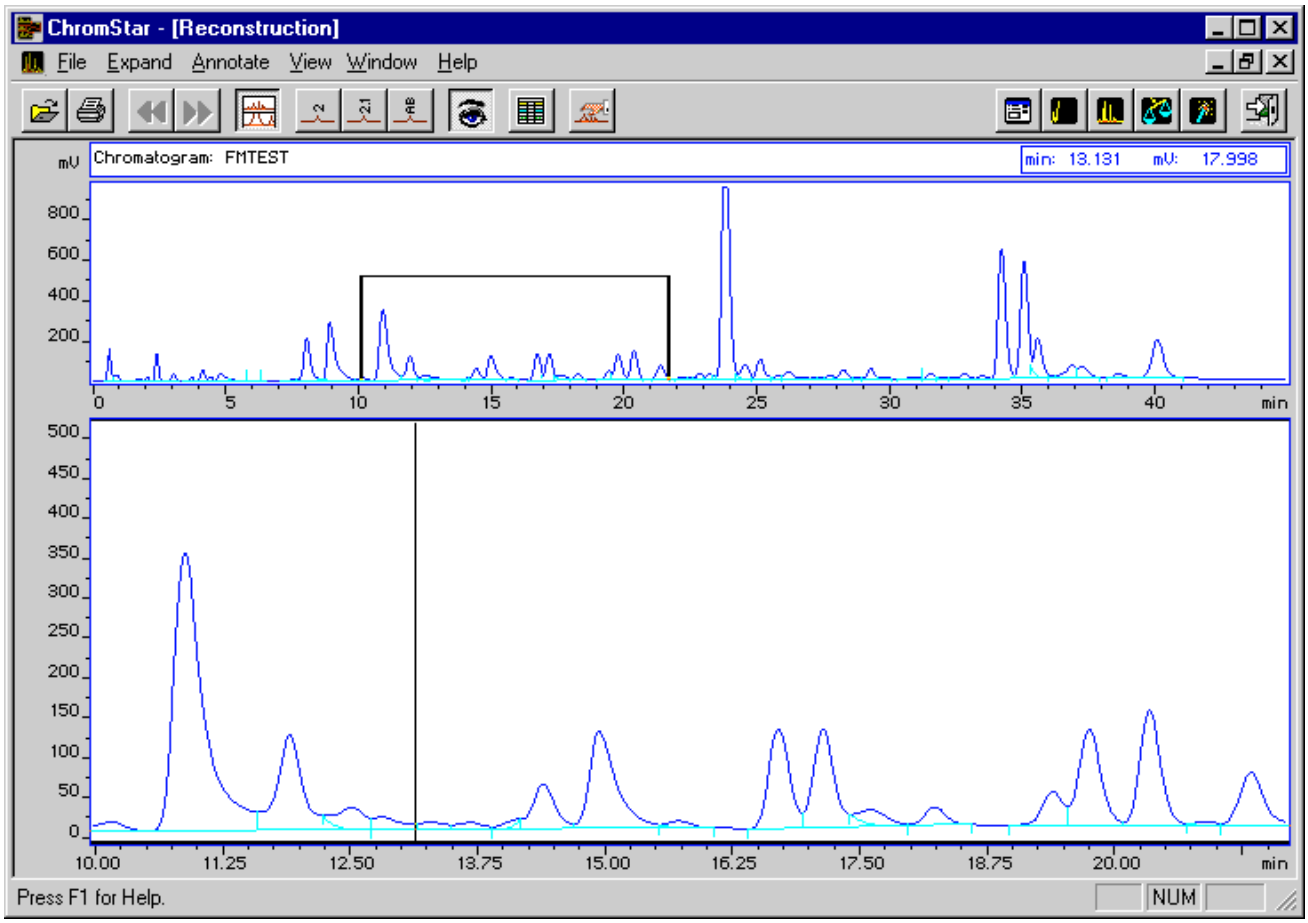
After clicking one line of the table and using the Shift- or Control key the lines marked in this way can be copied into the clipboard by using the *Copy* key. These lines can now be entered by using the *Paste* key in ***Edit Files, Calculation*** in the peak table. The box disappears when OK is clicked.



Show Comparison Chromatogram shows or hides the comparison chromatogram. The entry CompBase=1 in the section [Reprocess] in CHRST32.INI displays the peak baselines as well.

Split divides the chromatogram display window in two areas with a 1:2 proportion. The upper area is used to display the entire chromatogram, the lower area is used to display enlarged fragments of the chromatogram.

To do this first open a rectangle in the upper area. Its contents are displayed in an enlarged form in the lower area. The area covered by the rectangle is shown on a light background in the upper chromatogram.



A further enlargement is now possible it can be done in one of two ways:

1) The enlarged section displayed on the lower window sector can again be enlarged by opening a rectangle as described above and confirmed with the right mouse button. In the upper chromatogram the area on a light background is changed accordingly.

2) A new section can be defined in the upper chromatogram.

Clicking the upper chromatogram with **the right mouse button** allows you to erase the section.

The following operations function slightly different in the *Split* window:

Files, Print always prints out the section of the chromatogram which is displayed on the lower sector of the window. If the lower sector is empty the entire chromatogram will be printed.

Files, Copy transfers the part of the chromatogram in the lower sector of the window to the clipboard.

View, Show Values displays the retention time and the detector signal at the current position of the cursor in the lower sector. *Show values* can only function when a section is displayed in the lower screen

Results displays only the results list of the lower section of the screen.

Clicking again ***View, Split*** the split representation is cancelled .

After clicking ***Show Values*** a field is displayed in the status line above the chromatogram in which the retention time and the detector signal of each data point in the chromatogram are displayed. The vertical white line shows the position of the mouse cursor. Moving the mouse across the chromatogram makes the changing retention time and signal values visible in the view box. Clicking *Show values* or the button again causes the box to disappear.

Show Gradient. In the chromatogram representation the gradient used when recording the chromatogram can be shown (only possible when the pump is controlled via ChromStar and the gradient is entered in the LC Procedure file). On top of the chromatogram the color references to the solvents A – D are shown. For the print-out of the gradient the item *Show Gradient* must be marked in the object *Chromatogram* (c.p. Report-Editor manual).

Audit Trail opens a list showing all events which have happened during recording the chromatogram with date and time. In the simplest case *Start Chromatogram* and *Stop Chromatogram* are mentioned here.

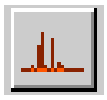
4.4.2 Reprocess - Integration

With ***Integration*** chromatograms can again be integrated.

After clicking the menu point ***Integration*** a submenu appears with the points ***Reintegration*** and ***Manual-Integration***. With *Reintegration* the evaluation is carried out with a data handling file containing the integration parameters. With *Manual Integration* peak recognition is done manually.

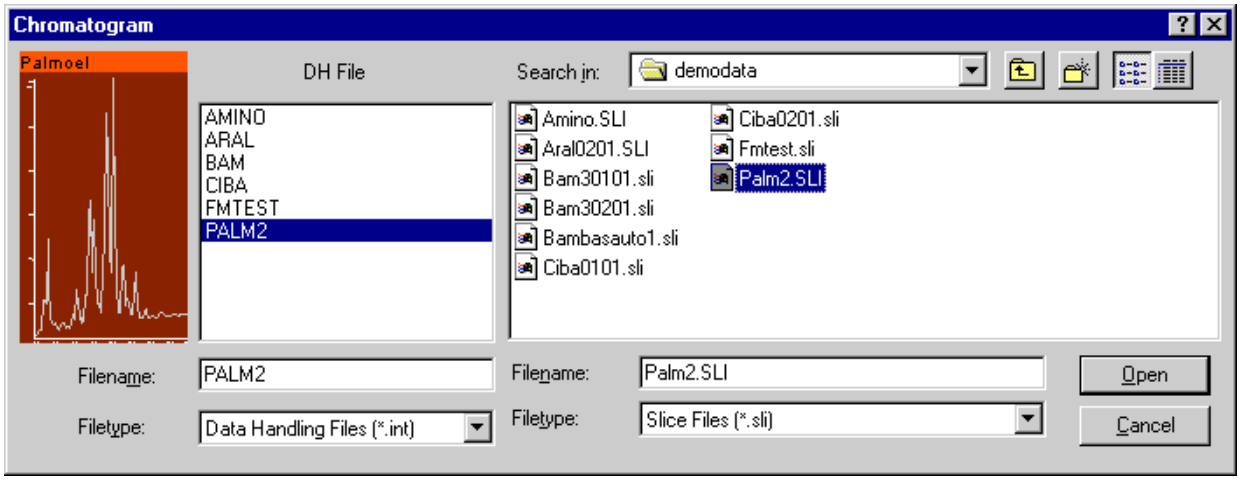
Both methods are described in the next chapters and in sections 5.2.1. and 5.2.3.

4.4.2.1 Reprocess - Reintegration



Using ***Reintegration*** chromatograms are re-integrated with a data-handling file. The result of the integration is stored in a temporary file with a name where the first character of the original file name is substituted by a ~ sign. The ~ -files (.RPT files and .SLI files) are shown in the file select box. On leaving ChromStar these files are deleted. The result of the integration can be saved in the original report file – a warning appears that the file is overwritten – or in a new file. ~-Files in Reintegration are immediately deleted by the entry DestroyTemp=1 in [Reprocess] in CHRST32.INI (DestroyTemp=0: the files are deleted on leaving ChromStar).

When the submenu point ***Reintegration*** is selected, a dialog box appears in order to select the chromatogram to be integrated and the data handling file.



A preview of the chromatogram and the *Sample identifier* - if any was entered when recording the chromatogram - appear in the box to the left.

Exit the dialog box with Open. After this the reintegrated chromatogram is displayed in the complete application window.

If a data handling file has been used for reintegration which has an existing calculation file defined against *Calculation*, then also the quantitative evaluation takes place according to the method defined there.

An enlargement can be made by setting up a rectangle with the mouse (cp. p. 4.4-5).

The menu bar now displays the menu points:

File Expand Annotate View Window Help

The ***File*** submenu offers various options for file processing.

The menu points are fully described in section 4.4.1 and will therefore only be mentioned here briefly.

Open returns you to the chromatogram and data handling file selection.

Open Previous selects the chromatogram, in a series of chromatograms, with the next lower number at the end of the chromatogram name.

Open Next opens the chromatogram with the next higher name..

Both menu points are light grey when no chromatogram with a higher or lower number is available.

With ***File Information*** details concerning the chromatogram are displayed.

Show Peaks from File opens a selection box from which an existing Report-File can be selected. The results list of this file appears on the screen.

Print permits the print-out of a chromatogram.

With ***Printer Setup*** printer settings like resolution, paper size, portrait or landscape orientation can be changed.

Copy opens a further submenu with the options *Chromatogram* and *Report* which allow you to select whether to copy the chromatogram or the results list into the clipboard.

Using ***Save*** the re-integration can be saved. A warning appears that the original report file will be overwritten.

Using ***Save as...*** the report can be saved under a new name. Automatically the chromatogram will be saved under the same name.

Using ***Revive*** an unfinished chromatogram (e.g. caused by a computer break-down) can be revived.

Export saves the chromatogram in a WINDOWS-meta-file (.WMF) after having selected an appropriate print report template. Thus the chromatogram can be presented in high resolution as vector grafic or in a desk-top-publishing program.

Reconstruction switches to the application Reconstruction with the reconstructed chromatogram.

Manual Integration opens the application **Manual Integration** and offers the possibility to execute a manual correction of the integration. The selected chromatogram is displayed in the upper section of the screen. The peak groups defined with reintegration are transferred to manual integration and can for instance

be erased there with *Delete*. The further execution of manual integration is described in section 4.4.2.2.

Exit Reintegration closes the application **Reintegration**.

Expand allows you to select a section of the entire chromatogram. After clicking *Expand* the submenu appears with the points ***Reference Window, By Values...*** and ***Default***.

Reference Window, Auto range allows you to construct a rectangle in the entire chromatogram in order to select a section. In the line above the chromatogram the message appears "Select rectangle to zoom" (cp. 4.4.1). The selected section is automatically normalized to the highest peak.

The submenu point ***By Values...*** allows you to zoom out by entering the time and mV values in a dialog box. Here *Auto Range* can be marked to in order to normalize the selected section to the highest peak.

The values in the dialog box always correspond to the current size of the chromatogram. These are also updated after using the submenu point ***Reference Window*** to zoom out.

Default returns to the display of the entire chromatogram.

Annotate,Automatic allows the user to mark peaks in a chromatogram by numbers, by retention times, or by names.

When ***Peak Numbers*** is clicked, the chromatogram with its peaks numbered is displayed.

Clicking ***Retention Times*** annotates the retention times above the individual peaks (cp. p. 4.4-6).

To annotate ***Peak Names*** it is necessary to have defined them in the calculation file which is specified in the data handling file used in the reintegration.

The submenu point ***Manual*** allows you to write individual comments horizontally or vertically on the chromatogram.

The annotation appears only on the current screen presentation, on the print-out and on the presentation transferred by means of *Copy*.

The menu point ***No-Annotate*** deletes an existing annotation.

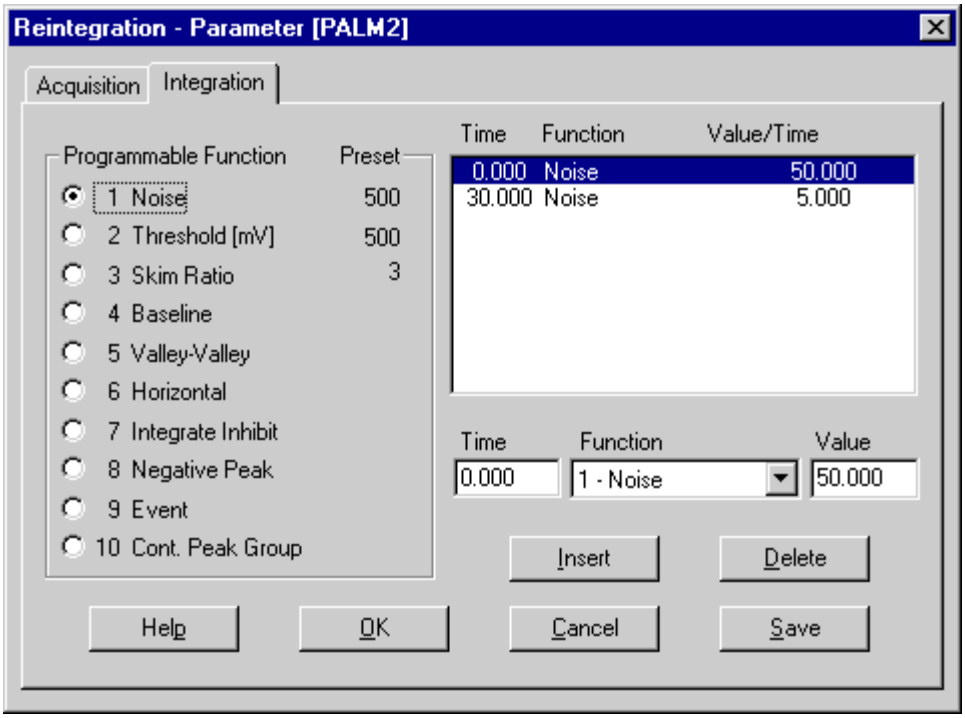
After clicking ***View*** the sub-menu ***Param, Results, Show Comparison Chromatogram, Split, Show Values, Show Gradient*** and ***Audit Trail***. appears.

The menu point ***Param.*** (Parameter) permits direct modification of the integration parameters. After clicking the menu point the data handling file appears as a dialog box.

The integration parameters can now be modified as described in 4.1.2.2. Exiting with OK starts the new calculation and the chromatogram is shown with the new integration results.

The card index Acquisition can be used to access the first page of the Data-Handling-File so that the parameters of this page can be changed.

Cancel aborts the calculation with the modified parameters.



With *Save* the changed parameter values are stored in the data handling file; otherwise they are lost after leaving the reintegration.

With ***Results*** the results list is displayed on the screen. The table contains peak numbers, retention times, peak areas and peak heights, the result of the calculation under Abs,/Rel. and peak names. The scroll bar at the right of the table allows you to scroll through the list if it is longer than the screen page. Marked peaks can be copied into the clipboard with the *Copy* key (cp. p. 4.4-6). The box disappears when OK is clicked.

Show Comparison Chromatogram shows or hides the comparison chromatogram. The entry CompBase=1 in the section [Reprocess] in CHRST32.INI displays the peak baselines as well.

Split Split divides the window displaying the chromatogram into two sections. The upper part displays the entire chromatogram; the lower enlarged sections of it as described in 4.4.1.

With ***Show Values*** a field is displayed in the status line above the chromatogram in which the retention time and the detector signal of each data point in the chromatogram are displayed.

Show Gradient. In the chromatogram representation the gradient used when recording the chromatogram can be shown (only possible when the pump is controlled via ChromStar and the gradient is entered in the LC Procedure file). On top of the chromatogram the color references to the solvents A – D are shown. For the print-out of the gradient the item *Show Gradient* must be marked in the object *Chromatogram* (c.p. Report-Editor manual).

Audit Trail opens a list showing all events which have happened during recording the chromatogram with date and time. In the simplest case *Start Chromatogram* and *Stop Chromatogram* are mentioned here.

4.4.2.2 Reprocess - Manual-Integration

Selecting the menu point ***Manual-Integration*** accesses a part of the program which allows manual peak recognition.

The chromatogram to be integrated is selected by means of the dialog box described in section 4.3.1. After selection the entire chromatogram appears in the upper part of the application window.

The menu bar contains the following points:

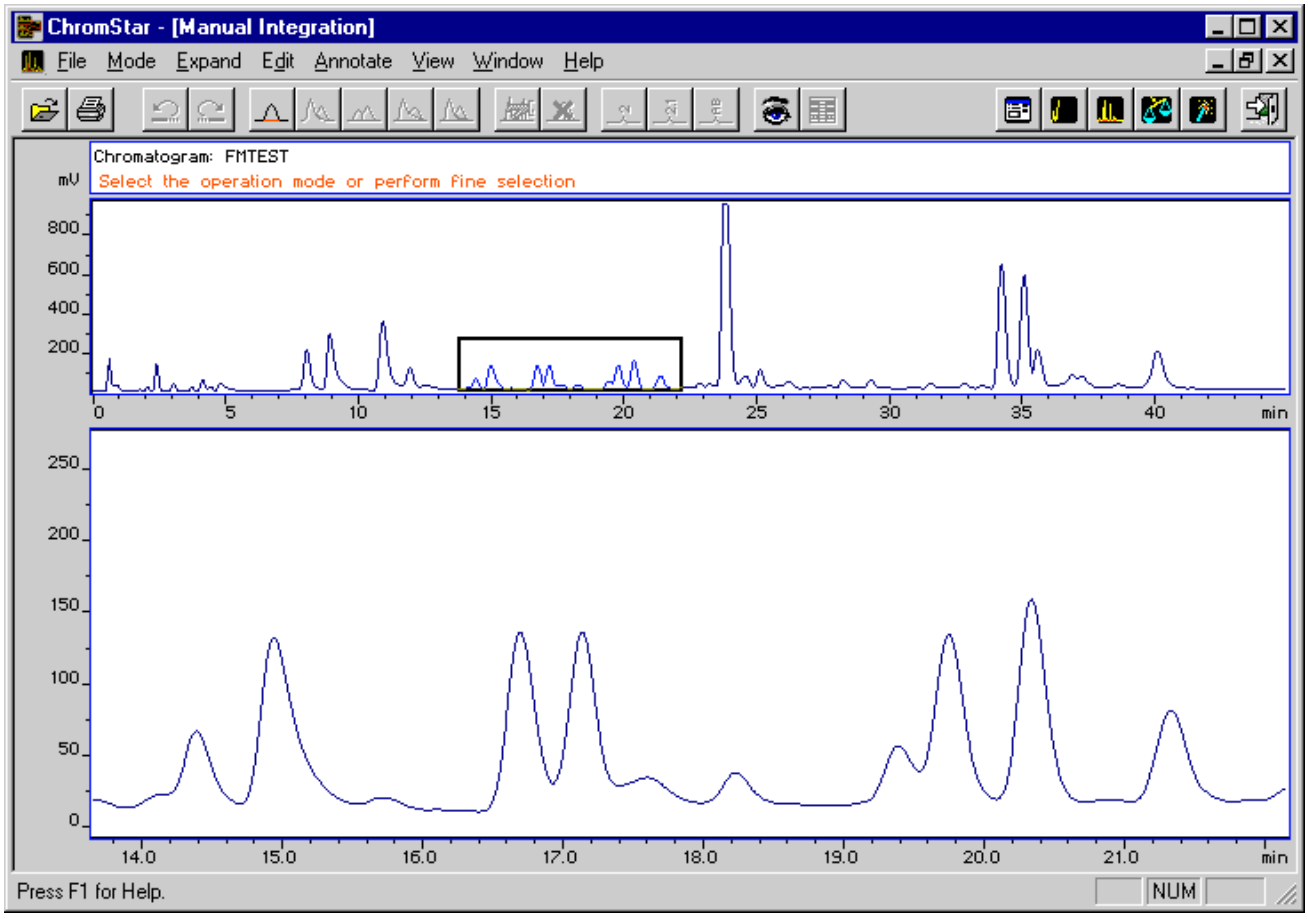
File Mode Expand Edit Annotate View Window Help

The buttons in the *Toolbar* to the left allow quick access to the main operations in this application. The are described together with their corresponding menu points.

In addition, a two-line information bar is visible. The first line contains the chromatogram name and a description of the actual work stage. The second line contains information to support the user in the handling of the individual menu points.

The manual integration is carried out as follows:

First the area in which the integration is to be made must be defined by constructing a rectangle as described in 4.4.1.



Before this you can use the menu point ***Expand*** to enlarge the displayed chromatogram by direct number entries.

With *Auto Range* in the entry field *Expand by Values* the selected section is automatically normalized to the highest peak.

The chromatogram section selected is displayed in the lower part of the window in its enlarged form and in the upper part it appears on a white background. The information field displays the message "Select the operation mode or perform fine selection" , i.e. further enlargements of the expanded section can be made in the lower part with rectangles before the final processing.

Attention! Shift + ESC cancels an existing enlargement.

Further processing in Manual Integration is done via the menu points ***Mode*** and ***Edit*** which will now be described.

The menu point ***Mode*** is used to define the baseline and calculation mode.

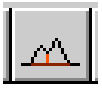
First selected is always the ***Tangential Baseline***. The information box displays the indication "Operation Mode: Tangential Baseline". To define the start point of the tangential baseline the mouse cursor is moved to the desired position, this point is fixed with a click on the left mouse button.

Moving the mouse arrow along the graph draws a white line. Click the left mouse button again at the desired end point of the baseline. The mouse arrow returns to the beginning where corrections can be made as necessary.

Clicking the right mouse button confirms the baseline.

The white line then changes to a green line on which start and end are marked. The baseline for a number of peak groups can be defined one after another. If no further peak separation is accomplished by one of the following menu points then the total area from baseline start to baseline end will be evaluated as one peak, even when the chromatogram is crossed by the baseline.

Exponential Baseline (not yet operational) is only used to separate a group of peaks positioned on the tail flank of a peak.



The second submenu point ***Valley*** is used to separate two peaks by constructing a perpendicular drop line through their valley. Before doing this the baseline for the whole peak group must have been defined as described above. Then the submenu point *Valley* is selected or the button is pressed upon which in the information bar the message appears "Operation Mode: Valley". After clicking the left mouse button a vertical white line appears at the cursor position in the area of a peakgroup with a defined baseline.

Move the mouse arrow to the deepest part of the valley between the peaks. A click on the right mouse button fixes a red drop line.

Clicking the left mouse button again brings back the white line, a new valley can now be searched and a drop line can be defined again with a click on the right mouse button.

Various valley points, also in different peak groups, can be defined one after another.

The submenu points ***Tangential Skim*** or ***Exponential Skim*** should always be employed for overlapping peaks when their heights are so different that the smaller peak can be regarded as being a rider peak on the descending flank of the main peak.

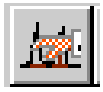
Before using this menu point a baseline must have been constructed for the whole peak group. The procedure is as follows:

Select one of the Skim methods from the submenu. The information bar now reads: "Operation Mode - Tangential/Exponential Skimming".

In the case of a ***Tangential Skim*** the start and end point have to be defined one after the other by moving the mouse cursor in the appropriate area of the chromatogram and clicking the left mouse button. This causes a white line to be drawn. Confirmation of the start and end points follows after clicking the right mouse button. After this a green line is drawn from the start to the end point. In the integration this area is subtracted from the main peak area.

The ***Exponential Skim*** function always leads from the graph curve to the baseline. The highest signal value always must be clicked first. After this a white line is drawn from the graph curve to the baseline, this line is dragged with the mouse to the lowest signal value. The end value is clicked and the cursor jumps back to the start point. Clicking the right mouse button confirms the skim function as a green line..

These functions also can be used consecutively for a number of peak groups.



Using ***Modify*** baselines, perpendicular or exponential peak separations can be changed afterwards. The cursor appears as an outstretched hand. As soon as the cursor is in a position where a change can be carried out it appears as a gripping hand. The information line shows which kind line will be changed e.g. *Baseline*, *Valley* or *Tangential*/*Exponential Skim*.

In the following the procedure for changing a baseline is described:

By clicking the left mouse key the baseline appears as white line. It can now change ist porition by moving the mouse. Clicking again the left mouse key moves the cursor to the other end of the baseline. By clicking the right mouse key the change is confirmed, the baseline appears in green.

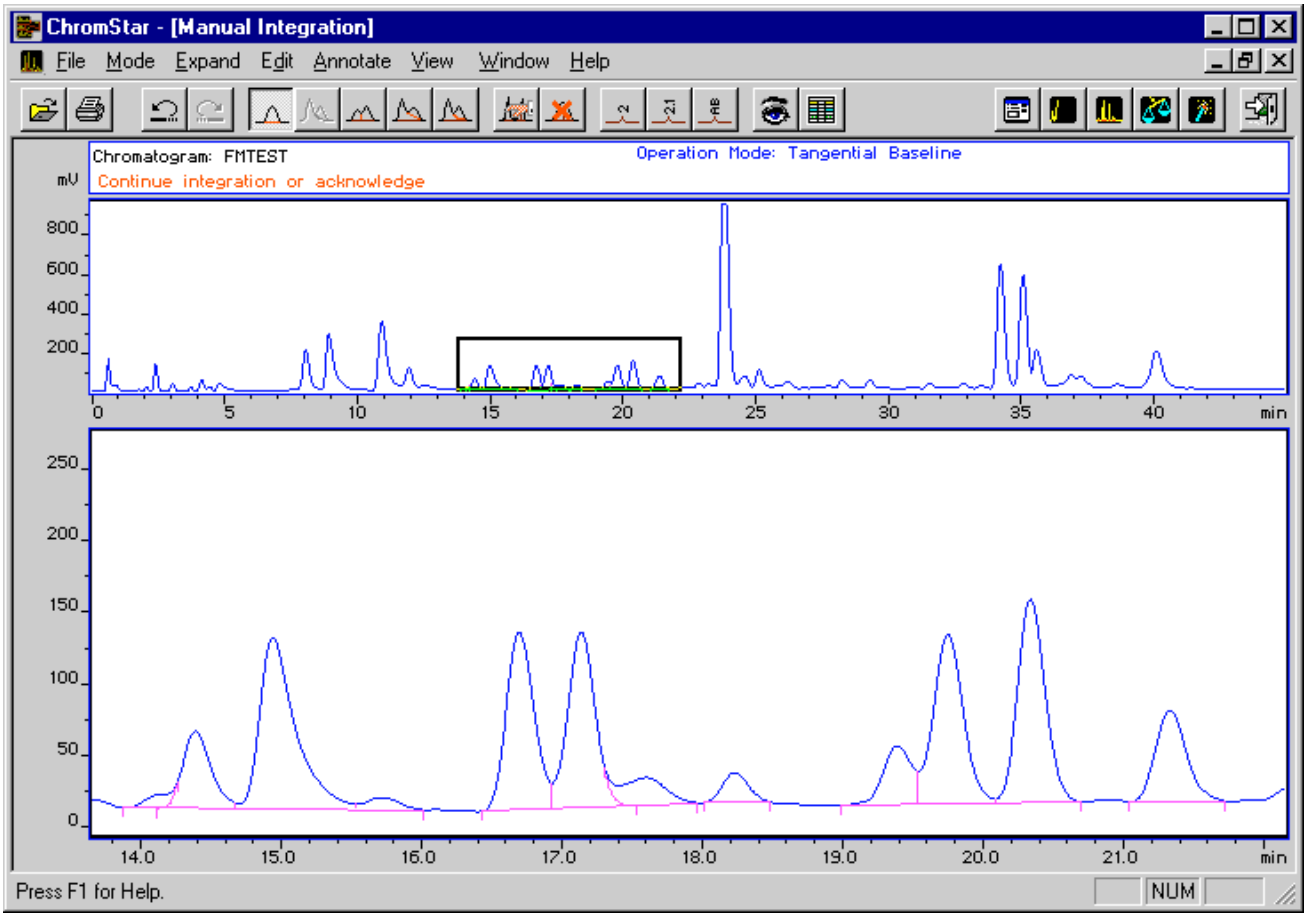
In order to change the perpendicular the left mouse key is clicked, the line is moved. By clicking the right mouse key the change is confirmed.

When the cursor appears as an outstretched hand the right mouse key switches from the *Modify* function into the *Delete* function.

Using ***Delete*** a baseline, a perpendicular or a skim can be deleted. The cursor appears as arrow with a cross. As soon as the cursor is in a position were a green line can be deleted it appears as arrow with a rubber. The information line shows which kind of line will be deleted, e.g. *Baseline*, *Valley* oder *Skim*. By clicking the left mouse key the line is deleted.

The integration of the peaks defined with ***Mode*** is effected by moving the mouse into the light area of the upper chromatogram and clicking the right mouse key. This transfers the graphic representation of the peak evaluation from the lower window to the upper. At the same time the light area disappears. Now a new area of the chromatogram can be selected and defined with a rectangle. The peak definition can again be made in the lower sector of the chromatogram as described above.

Negative peaks also can be integrated by defining a baseline. Their areas are shown as positive values.



Operations can be made undone or be re-activated by the menu ***Edit*** and ist sub-menu.

The menu point ***Undo*** can only be chosen when a section of the chromatogram is presented in the lower part of the display and after a manual peak separation was carried out. The operation carried out last will be deleted. The separation line in the display disappears. In this way several operations can be made undone stepwise.

The menu point ***Redo*** reactivates the operations stepwise. *Redo* can only be chosen, when *Undo* was used before.

The other menu points are fully described in section 4.4.1 and will therefore only be mentioned here briefly.

File has the following sub-menu:

Open... to select another chromatogram.

Open previous selects the next lower numbered chromatogram.

Open next selects the next higher numbered chromatogram.

File Information... displays various file information.

Comparison Chromatogram loads a comparison chromatogram.

Show Peaks from File... displays a Report-File.

Print... prints the chromatogram and/or result table.

Printer Setup... opens the printer setup window.

Copy copies the chromatogram or result table to the clipboard.

Save saves the evaluation in the Report-File.

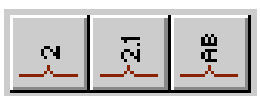
Save as... allows the chromatogram to be saved under a new name. At the same time the corresponding report file containing the result of the manual integration is created. ***Reconstruction*** transfers the chromatogram to the application **Reconstruction.**

Reintegration opens the dialog box "Select chromatogram and DH-file" which allows after selection of a Data-handling-file a quick transfer to the application Reintegration (cp. 4.4.2.1).

Exit Manual-Integration quits this program item.

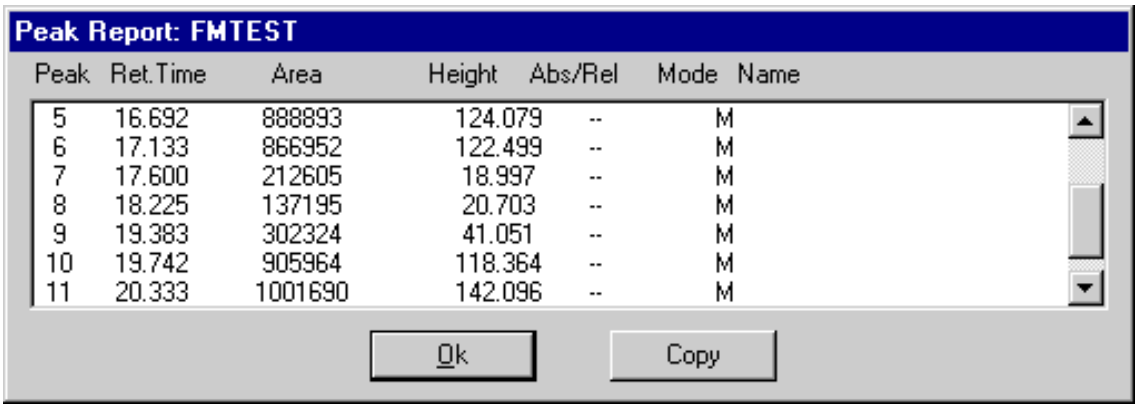
Expand permits the enlargement of the upper chromatogram by number entries (***By Values***) or to restore its original display (***Default***).

Annotate with its submenu points ***Automatic (Peak Numbers***, ***Retention Times, Peak Names), Manual*** and ***No Annotation*** operates only on evaluated peak groups in the main chromatogram. An enlarged part of the chromatogram in the lower section can only be annotated by means of ***Manual***. Annotation in the lower sector is possible as soon as integrated peaks are existing, these can in addition be annotated manually.



View offers the sub-menu procedures:

Results displays the results on the screen. The peaks obtained by manual integration are marked by an M. under *Mode*.



Show Comparison Chromatogram shows or hides the comparison chromatogram. The entry CompBase=1 in the section [Reprocess] in CHRST32.INI displays the peak baselines as well.

With ***Show Values*** the retention time and the corresponding detector signal at the cursor position in the lower sector of the chromatogram are displayed in a box in the information bar.

At the same time the submenu points of *Mode* for defining peaks can be accessed. *View* can only be accessed when a section is displayed in the lower sector.

Audit Trail opens a list showing all events which have happened during recording the chromatogram with date and time. In the simplest case *Start Chromatogram* and *Stop Chromatogram* are mentioned here.

4.5 Reprocess - Calculations

Quantitative Calculations, Calibrations, Comparisons, Batch Operations

This program part allows you to carry out a quantitative recalculation of a stored chromatogram, to execute different ways of calibrating to make up a calculation file, to carry out batch operations on a series of chromatograms, to compare up to 10 chromatograms and file conversions. Reprocess - Calculations contains the following menu points:

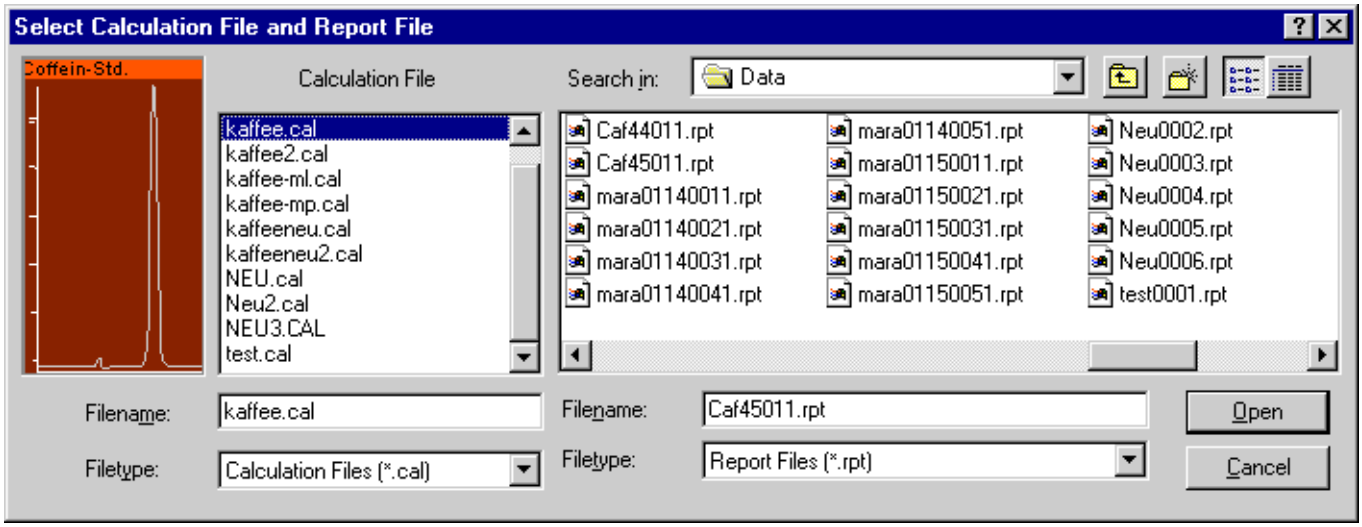
File Calculation Calibration Displ.-Chrom. Batch Convert Exit Window Help

The buttons in the *Toolbar* to the left allow quick access to the main operations in this application. The are described together with their corresponding menu points.

4.5.1 Reprocess - Calculation

Calculation performs a quantitative evaluation of a chromatogram with a calculation file using the Report-File (extension .RPT) that was generated during recording of the chromatogram. This Report-File contains the peak data obtained after integration of the chromatogram like retention times, peak heights and peak areas. In addition there must be a calculation file (extension .CAL) containing the response factors of peak areas or heights with the corresponding quantities (for calculation of response factors by calibration see cp. 4.5.2). The quantitative evaluation of a chromatogram in a current run is described in section 5.3.3.

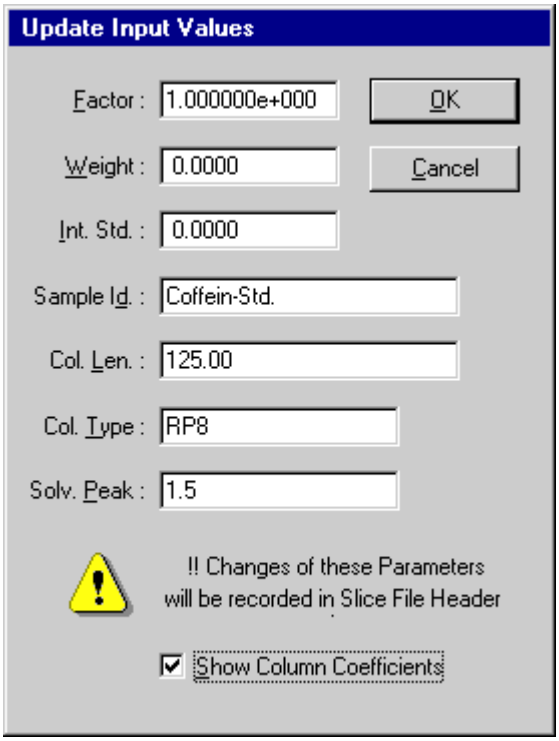
After clicking *Calculation* a dialog box appears for selection of a report and a calculation file.



After choosing a report file (.RPT) the corresponding chromatogram appears together with the *Sample Identifier* in the box to the left.

After both names have been entered and this box quitted with Open, a box appears in which the values for Factor, Weight and Int. Stand. and Sample Id.can be updated (cp. sect.4.1.1.3). New values can be entered here for factor (multiplication), for the total sample weight and for the amount of internal standard in case a run with internal standard has been selected.

For the calculation of column coefficients the *Column Length* and dead time (*Solvent Peak)* can be entered here. These parameters as well as the column description (*Column Type)* can also be entered in the *Documentation Table* of the method file (cp sect. 4.1.1.4) before recording the chromatogram. The box is closed with OK, the calculation is carried out and the results table is displayed on the screen.



If the column coefficients are to be shown the box *Show Column Coefficients* must be marked. For more details concerning column coefficients calculation cp. sect. 5.2.6.

The results table show details of the peak data (Report) and calculation files used, the peak number, window number (Win, corresponding with peak number in the calculation file), retention time, peak area, response factor (Res.Fct.), the concentration in the units defined in the calculation file (Absolute), the weight percentage of the total sample weight if entered (Weight) and peak names for the peaks defined in the calculation table.

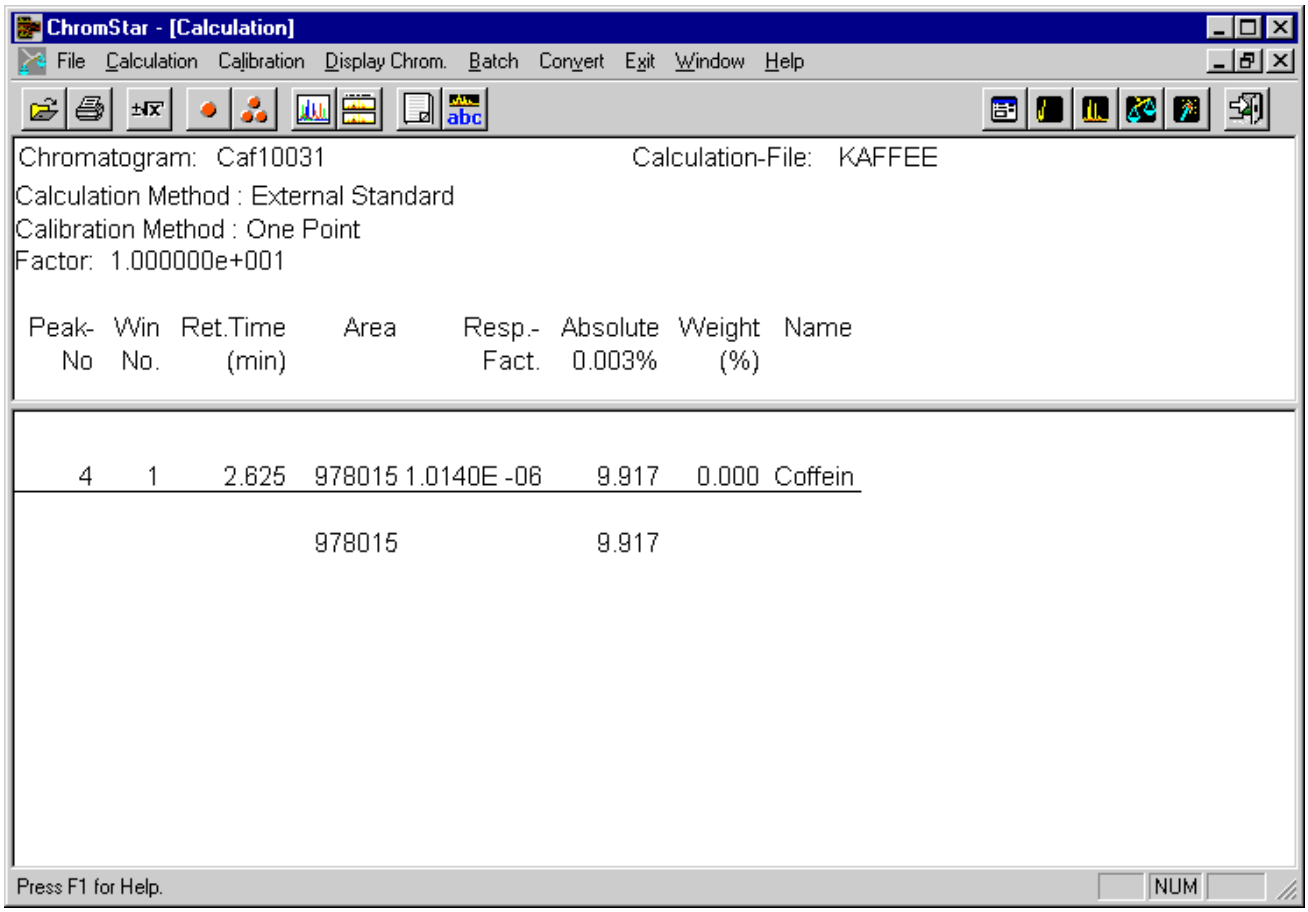
If more than one peak is found in a peak window, a peak selection can be made by entries in the Chrst32.ini file (s. page 4.1-26).

When a calculation is carried out after multi-point-calibration (average value of the response factors, entered as K1 in the Regression Table), the response factor in the results list shows the K1 value.

When a calculation is carried out after a multi-level-calibration, the response factor in the results list shows -----. For a correct documentation the print report template should contain the object Calculation File, in which all except the Regression Table can be removed.

In this display the grey line between table head and body can be moved up or down by pressing the left mouse key and moving the mouse as soon as the cursor appears as double arrow.

Quantitative calculations of a series of chromatograms can be carried out under ***Batch*** with *Mode = Calculation*.



File has a submenu with the following functions:

Open... (or ***Calculation***) allows a further report and calculation file to be opened for calculation.

Save saves the result.

Save As saves the result in a new file.

Load Multi Calibration shows a Multi Calibration once carried out and saved in a calculation file.

Print opens a dialog box , in which a print report template can be chosen (cp. p. 4.4-4) and a print-out be made (**8**). The print template should obtain the object Table (see also p. 4-6 in the Report Editor manual). Using the downwards arow after Fileformat the format of the print report template can be changed.

Printer Setup allows the changing of printer settings.

Copy transfers the results table to the clipboard.

4.5.2 Reprocess - Calculations - Calibration

Before starting a quantitative calculation as described in section 4.5.1 a calibration with one or several standard solutions must be carried out in order to determine the response factors for peak areas or heights with the sample amount.

In a one point calibration only one standard solution is used.

The multi calibration calculates:

- an average response factor out of the response factors obtained from one point calibrations after several injections of the same calibration sample or
- a calibration function representing the correlation between amount of a component and its peak area or height, this can be done after one point calibration with calibration samples that contain different amounts of the standard components.

The menu point ***Calibration*** with the sub-menu points

One-Point-Calibration
Multi-Calibration

permits various ways of calibration which are described in the following sections.

A further description can be found in section 5.3.1.

4.5.2.1 Reprocess - Calculations - One-Point-Calibration

For a one point calibration the Report-File of a calibration chromatogram and a Calculation-File are required. The Report-File contains the retention time, the peak area and the peak height for each peak as a result of the integration or reintegration of the calibration chromatogram. The calculation file is created as described in section 4.1.3. Page 2 of the calculation file (Peak Table) is used to define suitable time windows for each peak to be calculated. If more than one peak is found within these time windows the greatest peak will be used for calibration. If no peak is found in a defined time window the message appears:

"No peak found in time window -T1- to -T2-"

If one peak is found in more than one window the error message appears:

"Peak -Ret.-time- found in window -T1- to -T2- again"

When calibration samples are used with different amounts then the second page of the Calculation-File also contains the entries of these amounts under different Level-Numbers.

The Response Factor is initially set to 1.

In the calibration the Response Factor is determined by the equation:

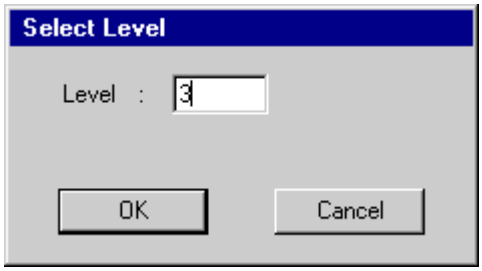
RF_i = Amt.-Std. / PKS_i

where

RFi	=	Calculated response factor of the i-th peak
Amt.-Std.	=	Amount of the standard entered against Amt. in Std. on page two of the calculation file
PKSi	=	Area or height of the i-th peak in the calibration chromatogram.

The calculated response factor is stored on page 2 (peak-table) of the calculation file against Resp. fact. and can be used later in a quantitative evaluation.

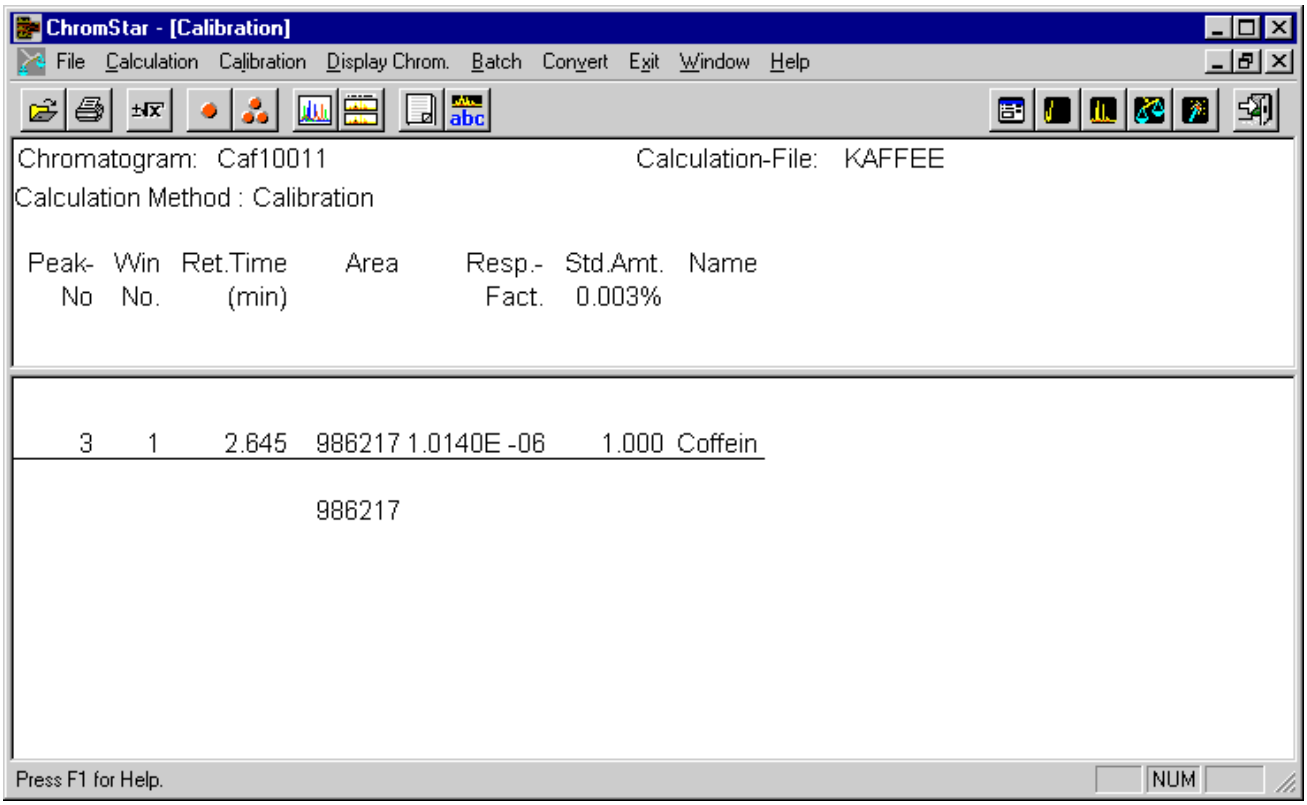
After clicking the menu point ***Calibration*** and its submenu point ***One Point Calibration***, a dialog box (cp. p. 4-65) is displayed in order to select the report and the calculation files. Selecting and clicking OK displays a second dialog box in which the number of the calibration sample, of which the injection generated the selected peak data file, is entered against Level.



The calculation starts after clicking OK.

The established response factors are entered into the calculation file being used and are available for quantitative calculations. In addition the results are entered in the Report-File. This then contains the amount, peak area or height, and response factor for each peak defined in the Calculation-File. The link between peak area and amount is used later in the Multi-Calibration (see next section).

After the calculation is finished a results list with new response factors (Res. Fct.) appears on the screen. The table also contains details of the amount of the substance in the standard solution and the name if this was defined in the peak table of the calculation file.



File has a submenu with the following functions:

Open... - or ***Calibration, One-Point-Calibration*** - allows selection of another Report and Calculation file.

Save saves the result.

Save As saves the result in a new file.

Load Multi Calibration shows a Multi Calibration once carried out and saved in a calculation file.

Print prints out the results table (**9**).

Printer Setup allows changing of printer settings.

Copy copies the table to the clipboard.

One-point calibrations with a serial of chromatograms can be carried out under ***Batch*** with *Mode = One Point Calibration.*

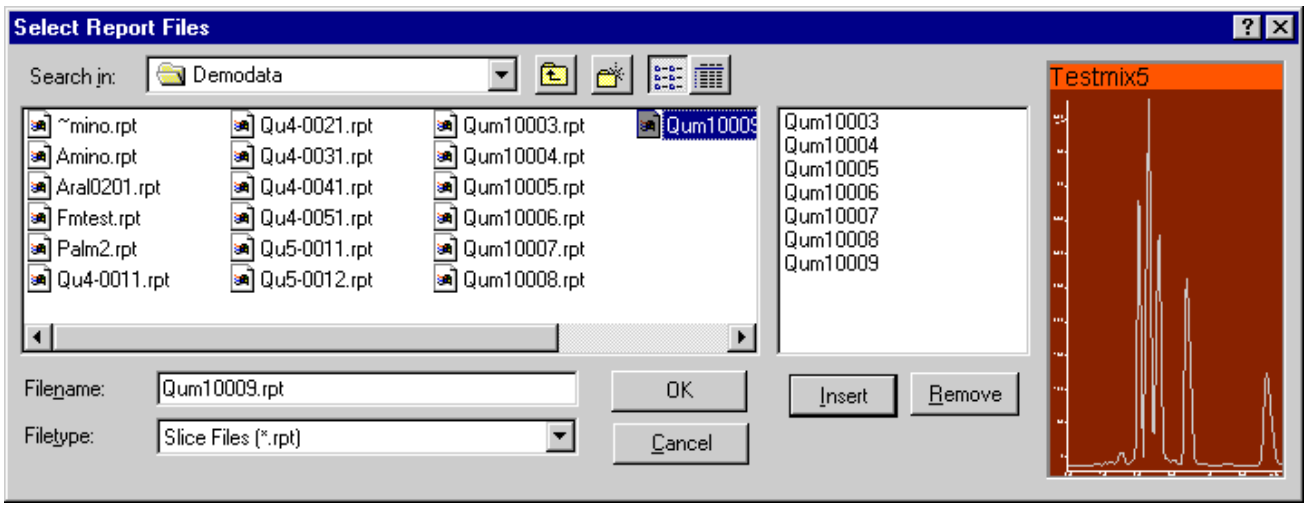
4.5.2.2 Reprocess - Calculations - Multi-Calibration

The submenu point ***Multi Calibration*** allows you to calculate the average value of the response factors after a series of one point calibrations or to calculate a calibration function and store this in a Calculation-File.

To carry out a multi calibration with average calculation, first record and integrate a number of chromatograms of one standard solution in order to establish the peak data. A calibration solution may contain more than one standard component. These peak data (in the Report-File) are used to carry out One-Point-Calibrations with a Calculation-File, that already contains the retention times and injected amounts, through this peak areas are linked to standard amounts and stored in the Report-File.

The peak areas stored in this way can be used to calculate average values.

After clicking ***Calibration*** and ***Multi-Calibration*** the Report-Files to be used for calculating average values can be selected (max.25) in the directory in the middle field which displays the content of the current directory. By double clicking or multi-selecting and *Insert* their names appear in the list to the right. A file can be deleted from the selected list by clicking its name in the field "Selected Files" and clicking *Delete*.



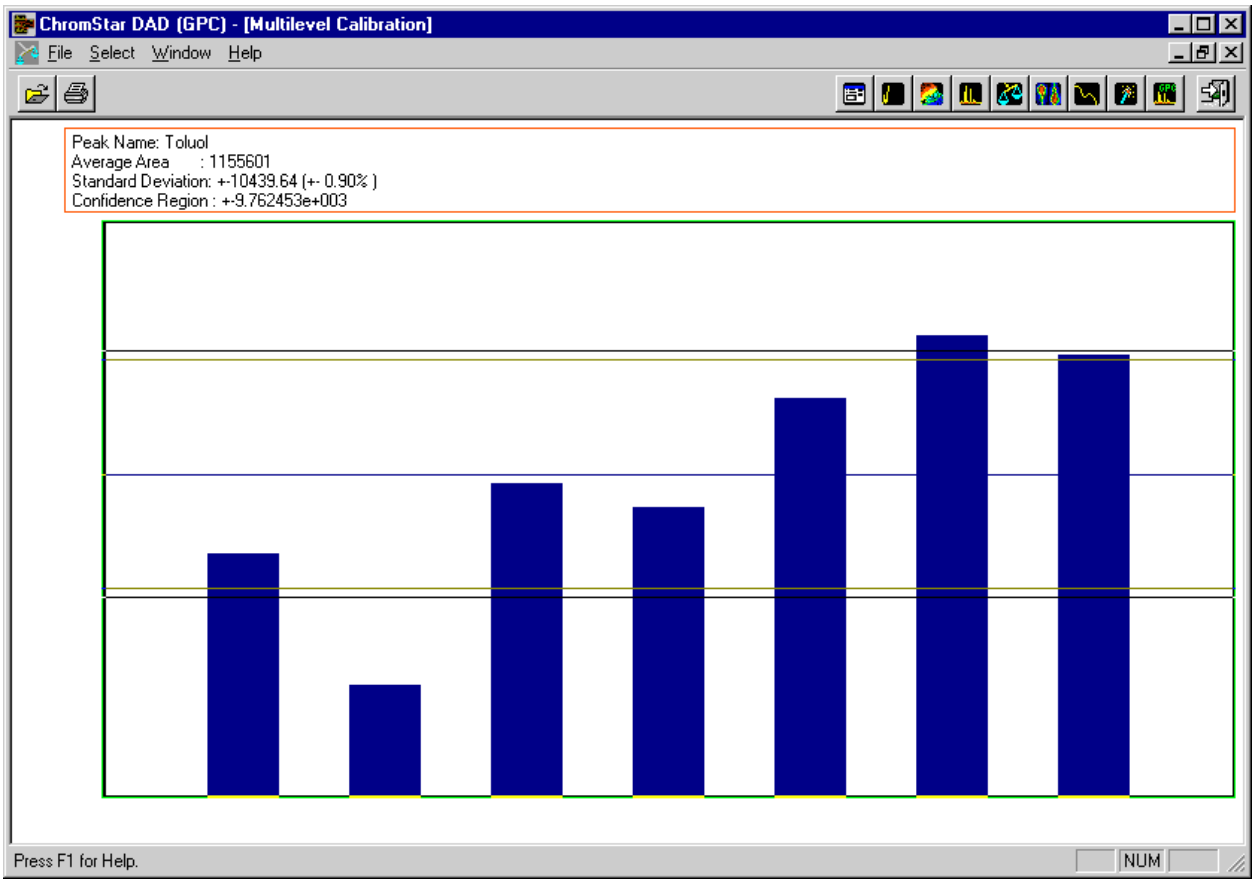
If a Report-File is clicked more than once and confirmed with OK the error message appears:

"Two identical Report-Files selected"

Attention! Only Report-Files generated by one point calibrations can be used for multi calibrations.

After correct file selection and clicking OK a graphical representation of the average calculation appears.

The yellow (here: blue) bars represent the peak areas of the individual runs, the horizontal yellow line in the middle represents the average value, the white (here black) lines represent the standard deviation and the blue lines the confidence range. The closer the blue lines are to the average value, the more reliable the calculation is. The confidence range decreases as more runs are made or if a few well reproducible runs are made.



In the field above the graph you will find first the components name of which the peak areas have been averaged; below this the average peak area as well as the standard deviation and the borders of the confidence range.

The menu bar contains the points

File Select Window Help

The menu point ***File*** and its submenu point ***Open...*** returns you to the selection of other Report-files and to a repeat multi calibration.

Load Multi Calibration shows a Multi Calibration once carried out and saved in a calculation file.

The ***File*** submenu point ***Save Results...*** is used to calculate response factors with the average peak areas, these response factors are then saved under K1 in the regression table of a calculation file. In the selection box the name of the calculation file appears which is defined in the selected Report File.

This name can be overwritten with a name that indicates that the calculation file contains an averaged response factor. ***Save Results...*** causes the averaged response

factors of **all** the peaks to be stored. The parameter *Regression for Calculation* at the first page of the Calculation File is automatically marked.

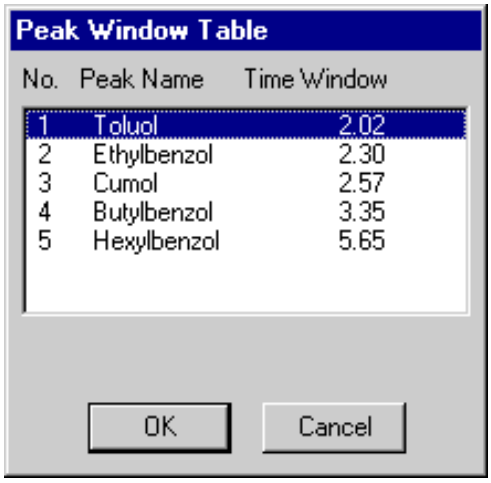
With ***Print*** and ***Results*** the results are printed (**10**). ***Graphic*** causes the graphical representation (**11**) to be printed.

Printer Setup allows the printer settings to be changed.

With ***Copy*** and ***Results*** or ***Copy*** and ***Graphic*** the results respectively the graphics are copied to the clipboard.

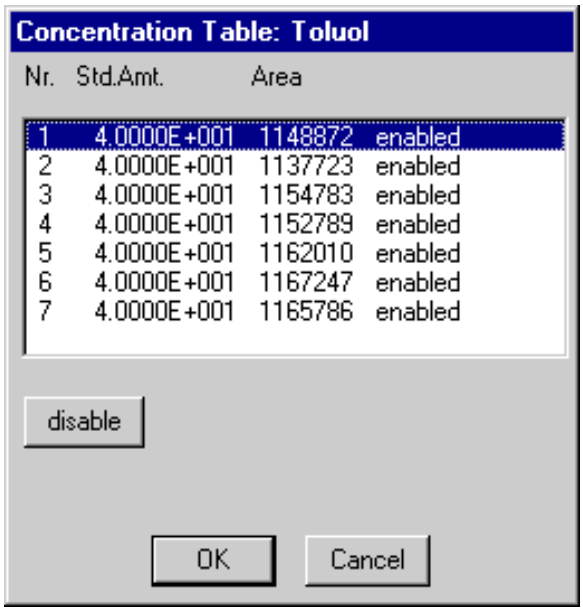
Exit returns you to the **Reprocess - Calculations** window.

Select contains the submenu points ***Get Peak-Table...*** and ***Get Concentration Table...***



Get Peak-Table... allows you to produce a graph of a further peak if more peaks of a chromatogram have been evaluated. After clicking *Get Peak Table* a window appears with the title Peak Window Table. This table lists all components contained in the standard solution and defined in the Peak Table of the Calculation File. Clicking a peak in this table and OK generates the graph of the averaging of the areas for this peak.

Get Concentration Table... displays a list of the peak areas used for obtaining the average value. One or more values in this list can be excluded from the calculation by *Disable* or restored by *Enable*.

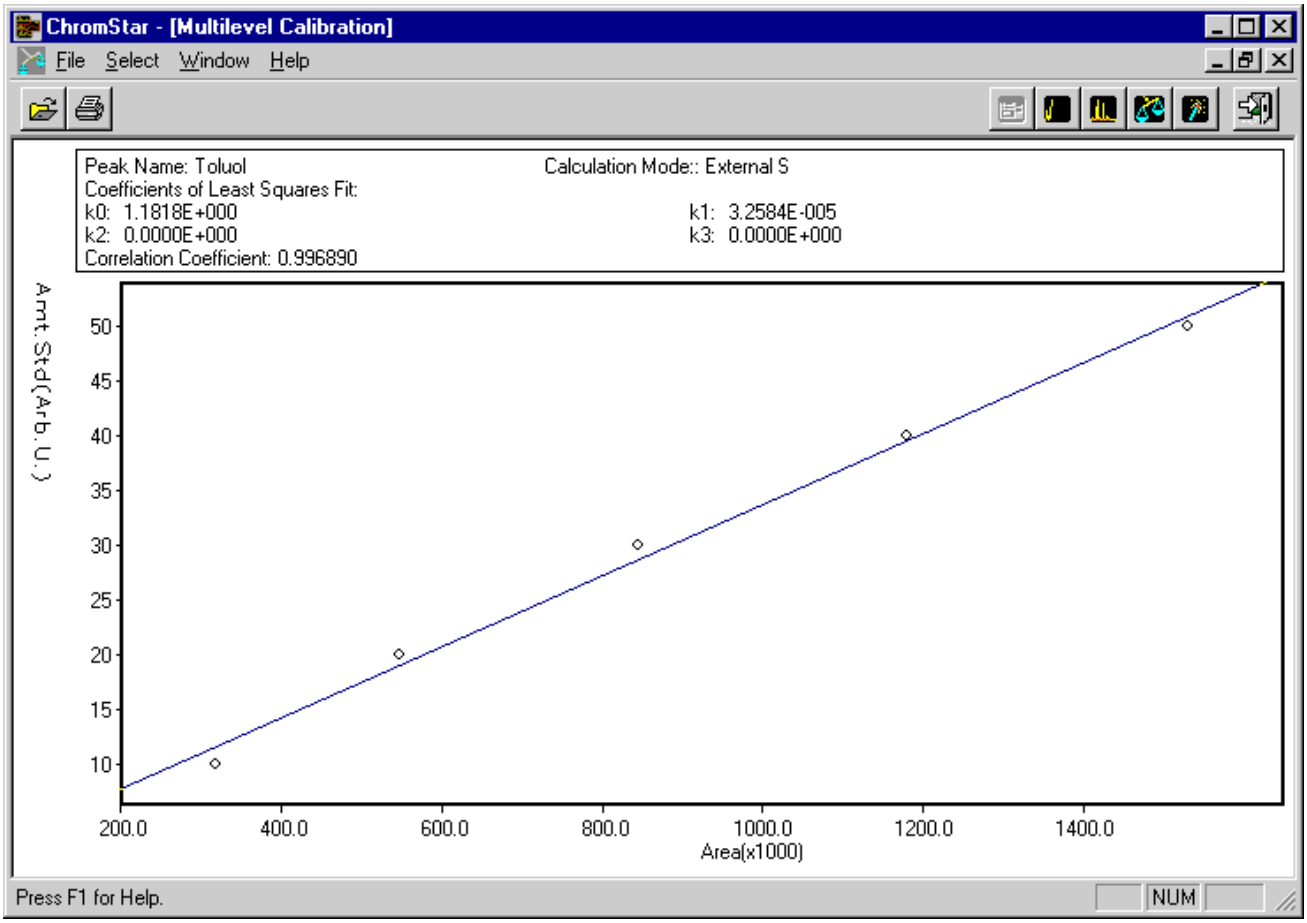


For calculating a calibration function (Multi-Level-Calibration) in Multi Calibration a number of Report-Files are necessary (max. 75, file extension .RPT, max. 15 levels) which contain the results of the One-Point-Calibrations with the standard solutions to be used for the calibration. The Report-Files, generated with the recording of the chromatograms, have to be changed in such a way that they are suitable for a Multi-Calibration. Therefore after recording the individual chromatograms they have to be evaluated in a one-point-calibration with the Calculation-File containing the exact amounts (as described in 4.5.2.1).

Attention! Only Report-Files generated by one point calibration may be used for multi level calibrations. A Report-File may not be used more than once.

After clicking into ***Calibration*** select ***Multi Calibration***. A dialog box appears for selection of the various Results-Files (cp. p. 4.5-7). After selecting the desired Report-Files by double clicking or multi-selecting and *Insert* their names appear in the list to the right. On clicking OK the calculation starts and the approximation function of the first order is displayed for the first peak using the peak areas or heights for the x axis and the quantities (entered under Amt. in Std. in the peak table of the calculation file, which are used to generate the Report-File) for the y axis. In addition, the coefficients of the regression function and the correlation coefficient are displayed. The form of the third order approximation is:

$$y = K0 + K1*x + K2*x^2 + K3*x^3$$



The menu bar contains the points:

File Select Window Help

File and ***Open...*** allows you to select alternative Report-Files for a Multi-Level-Calibration.

Load Multi Calibration shows a Multi Calibration once carried out and saved in a calculation file.

Using ***File*** and ***Save Results*** the regression function can be stored in a calculation file; default name in the entry box is that of the calculation file belonging to the first selected Report-File. This name should be overwritten with a name showing that this calculation file is obtained by a multi level calibration. The regression coefficients are entered on the third page of the calculation file. Regression coefficients of all peaks in the calibration solution are stored. In this newly-generated file the parameter *Regression for Calculation* on the first page is marked, owing to this the regression coefficients of the third page are used in later calculations.

If a calibration function of the second or third order is to be stored, first select the required order by clicking ***Select*** and ***Order*.** Then click ***File*** and ***Save Result*s**, enter a suitable name for storing the calibration function which is then available for further calculations. For each peak another order can be chosen.

Print and ***Results*** prints out the calibration table (**12**); ***Print*** and ***Graphic*** the graph of the calibration function (**13**).

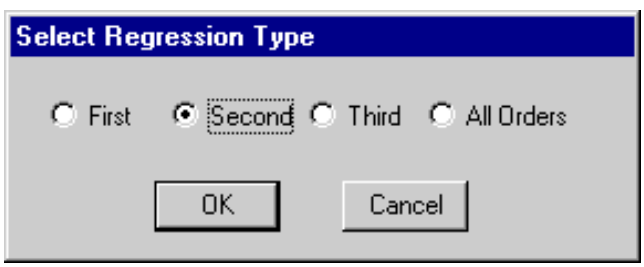
Printer Setup allows the printer settings to be changed.

Copy and ***Results*** or ***Copy*** and ***Graphic*** copy the calibration table respectively the graph to the clipboard.

Exit returns you to the **Reprocess - Calculations** window.

Select contains the submenu points ***Order, Get Peak Table...*** and ***Get Concentration Table***.

Clicking ***Order*** allows the permissible approximation functions - i.e. of the first, second and third orders or all together - of the calibration to be displayed on the screen. This screen display can be printed out with ***File, Print*** and ***Graphic*.** At least 5 Report-Files are necessary to calculate a third order approximation function. If all calibration functions (first to third order) are selected for presentation after clicking ***Order*** and the results stored via ***File*** and ***Save Results...***, only the coefficients of the first order of approximation will be entered in the regression table of the calculation file. For each peak another order can be chosen.

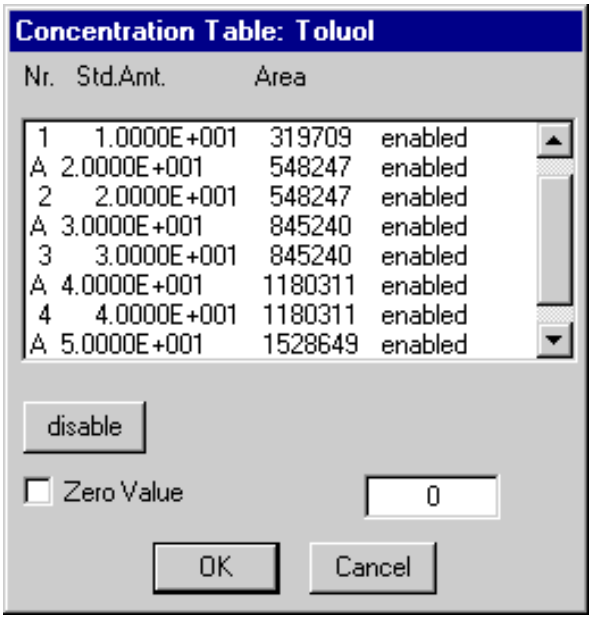


Clicking ***Get Peak Table...*** displays a list of all the peaks with their names and retention times (cp. p. 4.5-9). Clicking the respective line of a peak, which is then displayed on a black background allows the calibration function of this peak to be selected and displayed as a graph. *Select* and *Order* allows you to define another approximation order for this peak. It is therefore possible to use different approximation orders for all the peaks of a peak table.

Get Concentration Table displays a table with the concentrations of the individual components for each calibration run. In case of multiple injections of one calibration sample the average area is displayed as first entry above those of the individual runs. Individual values in the table can be marked with Disable. Then after clicking OK the calibration function is evaluated without this particular value. This allows you to exclude incorrect results from a calculation initially and later to recheck this concentration by re-recording the chromatogram.

In a multi calibration, where a series of standard solutions with different standard amounts is injected more than once, a regression without averaging the areas of the single injections can be carried out with the entry MultiCalNoAverage=1 in the section [Calibration] in CHRST32.INI. If there is no entry or the entry is 0, an average value of the single areas is calculated and then the regression is carried out using the average values.

When executing a multi level calibration the zero level can also be used as a calibration point. Click *Select* and *Get Concentration Table*... and the table with the calibration concentrations and corresponding peak areas appears in which you can now mark the Zero Value box down left. The value in the entry box at the right now reads 0. After clicking OK, the new calculated calibration curve appears in which zero is used as a data point and is drawn in the graph.

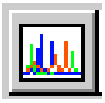


If the component to be evaluated is eluted together with an interfering substance at a constant level, a blind value may be entered in the entry box at the right once Zero Value has been clicked. This blind value must previously have been determined as a peak area by recording a chromatogram without the calibration component.

4.5.3 Reprocess - Calculations - Displ.-Chrom.

In the program section ***Displ.-Chrom.*** chromatograms can be displayed in different ways in order to be compared. The different possibilities, offered by the submenu points ***3D-Plot*** and ***Compare,*** to display chromatograms are described in the following sections.

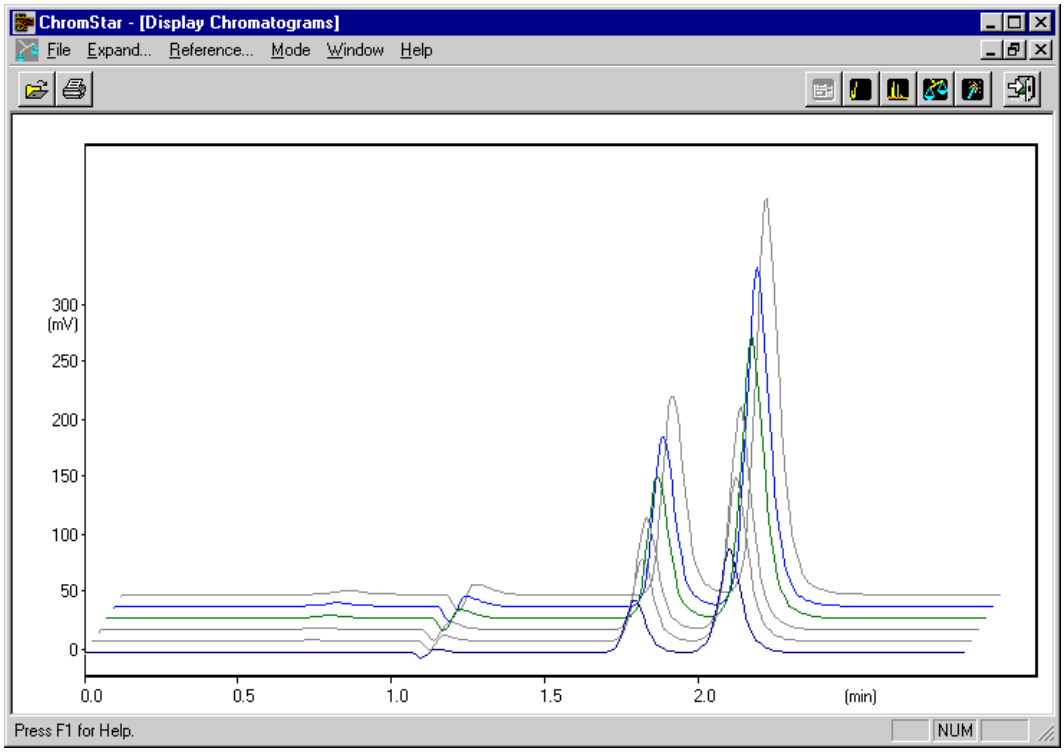
4.5.3.1 Reprocess - Calculations- 3D-Plot



Clicking the menu point ***3D-Plot*** displays a dialog box in which up to 10 chromatograms can be selected. A list of all directories is displayed on the left, in the centre the raw data files of all the chromatograms of the selected directory. The chromatograms are selected by double clicking or multi-selecting and *Insert*. If more than 10 chromatograms are selected and confirmed with OK the message is displayed:

"Too many data files"

After correct selection and clicking OK, the chromatograms are displayed in a three-dimensional form, the first selected chromatogram is at the bottom. The scaling of the x-axis is determined by this chromatogram. The individual chromatograms are displayed on the screen in different colours.



The menu bar contains the menu points

File Expand... Reference... Mode Window Help

File allows various file operations.

With ***Open*** further chromatograms can be selected to display on the screen

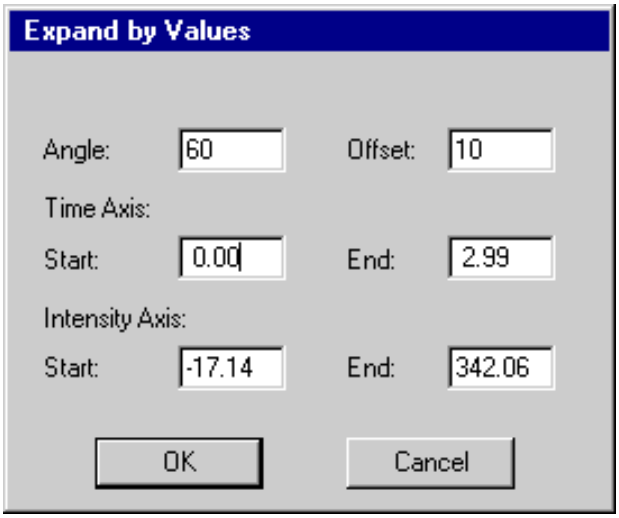
The menu point ***Print*** transfers the displayed sections of the chromatogram to the printer after selecting a convenient report print template (**14, 16**).

Printer Setup allows the printer setting to be changed.

Copy copies the screen display to the clipboard.

Exit returns you to the **Reprocess - Calculations** window.

Selecting the menu point ***Expand..*** allows you to modify the size of the Angle and the Offset value as well as the start and end times and the maximum and minimum signal value all of which influence the three-dimensional representation.



The following limits apply when selecting these parameters:

Angle = 0 and Offset = 0 gives a two-dimensional display of the overlaid chromatograms.

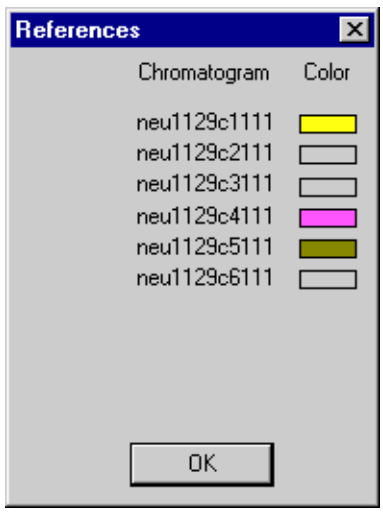
Angle = 90 and Offset >> 0 gives a two-dimensional display of the chromatograms stacked parallel along the y axis.

The Offset value determines the distance between the baselines of the individual chromatograms.

An enlarged section of the display of all the chromatograms can be made by constructing a rectangle which always has the lowest chromatogram as its base. This enlargement can be repeated at will. The original display of the full-length chromatograms is re-accessed by clicking the right mouse button.

Selecting the menu point ***Reference*** displays a dialog box which shows a list of the individual chromatograms and their colours used in the screen display. The box is closed with OK.

The colors in used for the different chromatograms can also be used in the print-out, when in ***Edit Files, Colors*** the *Print Colors (Channel 1 – 10)* are the same as the *Screen Colors (Channel 1 – 10)* and the object Multi Chromatogram (in the report print template .rpd) has the property *Use ChromStar Colors* marked.



The menu point ***Mode*** allows you to display the chromatograms in one of the four following modes:

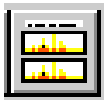
Standard: The entire chromatogram is displayed, i.e. the section of the three-dimensional display overlaid by another chromatogram is shown as well.

Whitewash: The section overlaid by another chromatogram is not displayed.

Data Point: The entire area of the chromatogram is displayed in colour, i.e. each individual data point is shown as a dash. If only one chromatogram is being displayed in enlargement, this mode provides a graph of the individual data-points.

Point Display: the data-points are displayed as dots.

4.5.3.2 Reprocess - Calculations - Compare

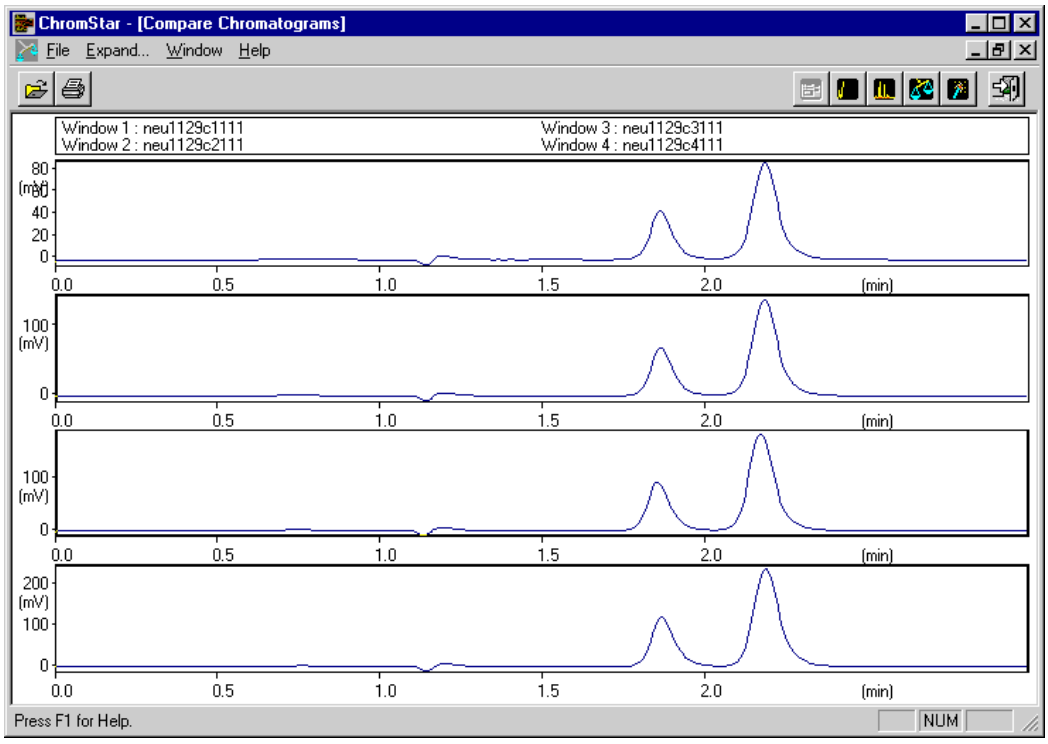


The submenu point ***Compare*** allows a stack of up to four chromatograms to be displayed each with its own time axis.

After selecting the menu point *Compare* a dialog box is displayed in which the chromatograms to be displayed are selected. The names of the raw-data-Files are selected by clicking them in the list at the centre, which displays the raw-data-files of the selected directory. The selection is terminated with OK. The chromatograms are displayed in windows on top of each other.

The menu bar contains the menu points:

File Expand... Window Help



A sectional enlargement in each chromatogram window is possible by constructing a rectangle. Clicking the right mouse button in the respective window re-accesses the full display of the chromatogram. Here too, a sectional enlargement can be further enlarged.

The menu point ***File*** allows various file operations.

With ***Open*** further chromatograms can be selected to display on the screen

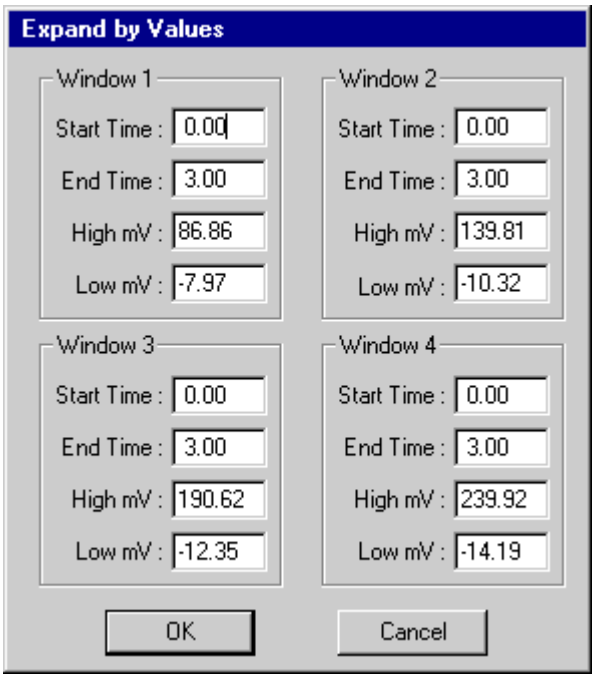
With ***Print*** a print-out is made after selecting a convenient report print template (**15**).

Printer Setup allows printer settings to be changed.

C*opy* copies the screen display to the clipboard.

Exit returns you to the **Reprocess - Calculations** window.

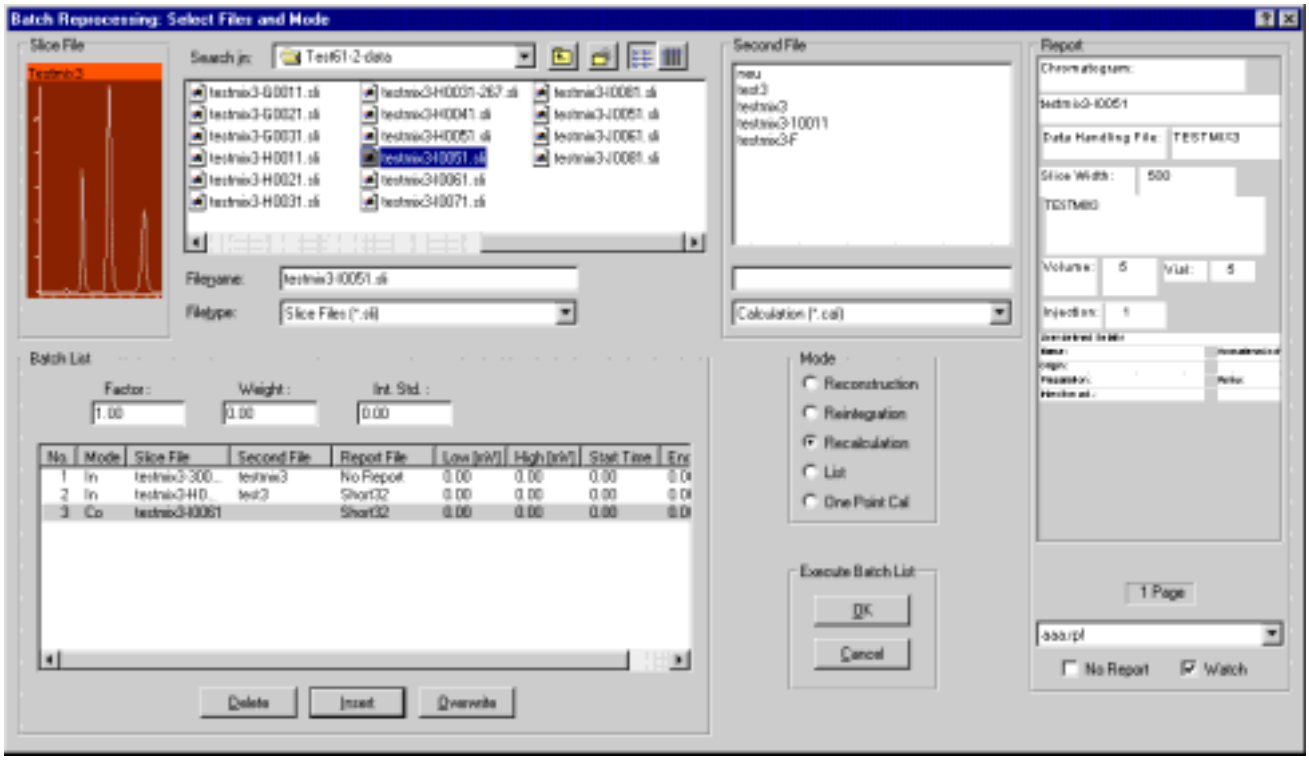
Expand... allows you to change the start and end time values (x-axis) and the mV minima and maxima (y-axis) for each window.



4.5.4 Reprocess - Calculations - Batch

The menu point ***Batch*** of the **Reprocess - Calculations** window allows the reprocessing of whole groups of chromatograms.

After clicking *Batch* a dialog box appears in which all operations to be carried out have to be defined.



The current directory is displayed after *Search in*, it can be changed via the downwards arrow to the right.

The chromatogram to be processed is selected under ***Slice File*** (the corresponding Report-Files must be available, there is no separate indication here).

Under ***Second File*** the Data-Handling-File required for reintegration or the Calculation-File required for recalculation are selected. The title of this list changes according to the selection made under *Mode*.

The following operations can be selected in the ***Mode*** field:

Reconstruction for presentation of chromatograms;

Reintegration for recalculation of peak areas;

Recalculation for quantitative evaluations;

List (as Reconstruction);

One Point Calibration for carrying out one-point calibrations

It is also possible to select a part of the y-axis of each chromatogram with the Low mV and High mV values. Start time and End Time allows the start and end time of

each chromatogram to be selected. The entire chromatogram is presented if all values remain 0.

When a recalculation is being carried out the values for *factor*, *weight* and *Int. Std*. can be re-entered.

For one-point calibrations the appropriate level number of the standard chromatogram must be entered under *Level*.

The type of print-out is selected in the field ***Report***. A print preview can be seen in the box to the right. With *No Report* no print-out is generated at all.

After selecting a chromatogram, the required operation, the necessary Data Handling or Calculation-File, the type of Print-out and the required section all entries are transferred into the ***Batch List*** with *Insert*. Chromatograms can be removed from the list with *Delete*. A scroll bar appears when more than 5 entries are made.

The chromatograms can be entered in the list quickly by double click. Selection of several chromatograms can be made by using Shift or Ctrl key.

The reprocessing is started after clicking OK in the **Execute Batch File** box.

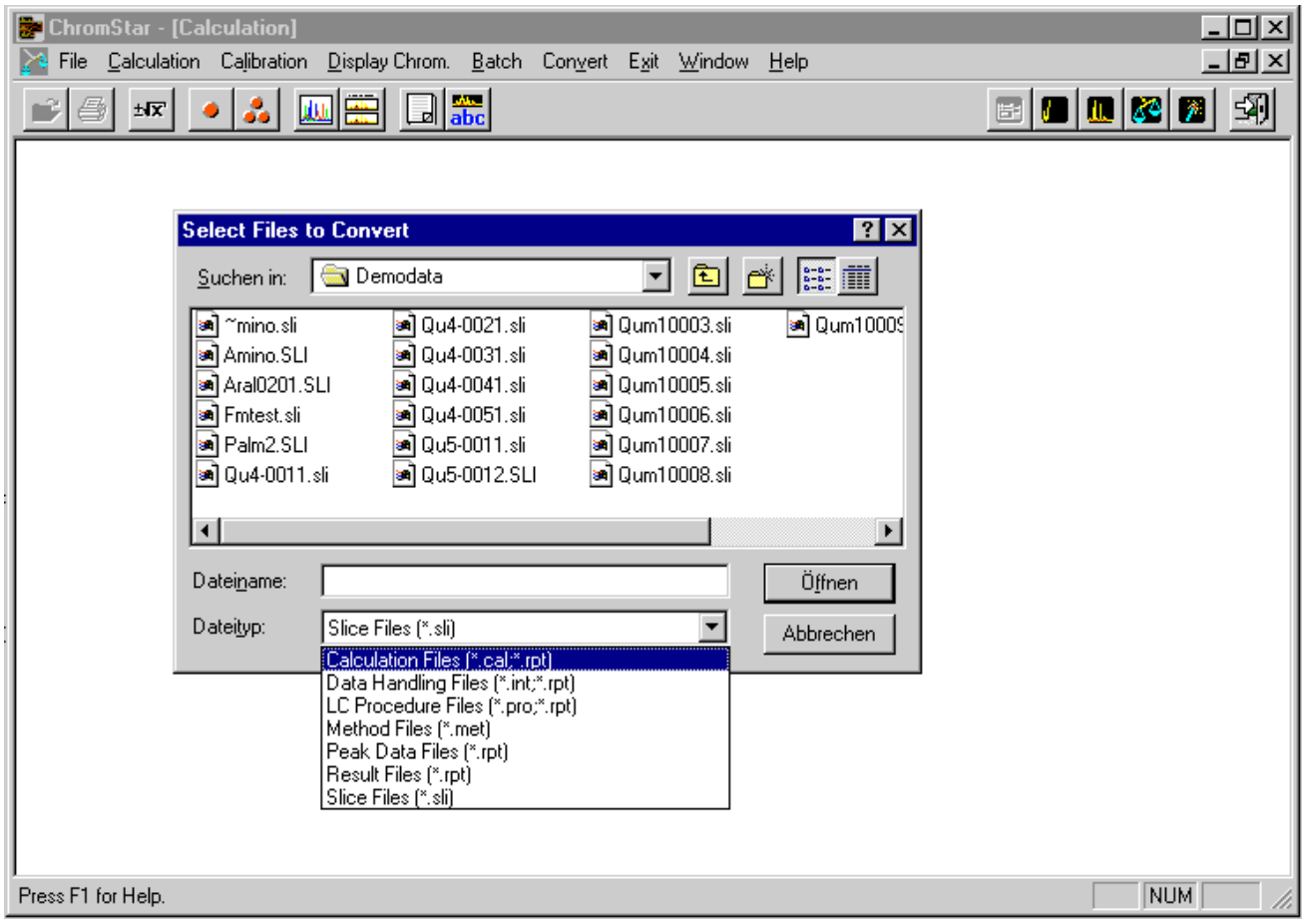
The results of the calculation are added to the Report-File as last entry.

In the **GLP mode** chromatograms are not overwritten when reporcessed. New files are created (chromatograms .SLI and report files .RPT) with inserted characters in the filename. After calculation _C_ is inserted in front of the last 4 characters (= current number of the chromatogram), after reintegration _I_ is inserted and after one-point-calibration _O_ is inserted.

4.5.5 Reprocess - Calculations - Convert

The menu point ***Convert*** in the **Reprocess - Calculations** window with its sub-menu ***To ASCII***, ***Slice File to Andi*** and ***Andi to Slice File*** enables you to convert ChromStar files in ASCII files und to convert ChromStar chromatograms (.SLI) into Andi files (.CDF) and vice versa..

After clicking ***Convert, To ASCII*** a dialog box appears in which the File type (List Files of Type, down left) and the names of the files to be converted can be selected by clicking and multiple marking. Only files of the same type can be selected for conversion. The marked file names in the list appear under File Name one after the other in such a way that only the last two are visible.



A maximum of 25 Files can be selected for conversion.

The original ChromStar files remain of course unchanged.

The conversion starts after pressing the *Open* key. The window is automatically closed. The converted files are in the same directory as the original files. They can be opened e.g. by the windows application *Notepad*.

Depending on the file type different file extensions are allocated to the converted files, they are summarised in the following table.

File type	ChromStar-File extension	ASCII-File extension
Raw data (Slices)	SLI	SLT
Calculation	CAL	CAT
Data Handling	INT	ITT
Method	MET	MTT
LC-Procedure	PRO	PRT
GPC Calibration	GCA	GCT
GPC Data Handling	GIN	GIT
Reportfile	RPT	
Peaks Data File after Integration		PKT
Result File after calculation with Cal.file		RET
GPC-Distribution-Plot		GDT

The content of the Data Handling, Calculation, LC Procedure, GPC-Data-Handling or GPC-Calibration files used when recording a chromatogram is stored in the report file. These files can also be converted. As ASCII file they get the name of the report file and the extension ITT, CAT etc.

Multiple file selection (according to WINDOWS) is done as follows:

Click the start file name, press the shift key and hold it depressed, click the end file name causes all files between starting and ending file to be marked.

Click the start file name, press the CTRL key and hold it depressed, click any other file names causes only the files clicked to be marked.

Slice File to Andi converts chromatograms into Andi files (.cdf).

Chromatograms can be saved in the Andi format (.CDF) at the same time as in the ChromStar format (.SLI + .RPT) with the entry DoAndi=1 in chrst32.ini in[Analysis].

Andi to Slice File converts Andi files into ChromStar kompatible chromatograms (.sli + rpt). The report file must be fitted by a reintegration.

4.8 Transform

The program module **Transform** allows various chromatogram transformations. The first or second derivative of a chromatogram can be calculated. A chromatogram can be smoothed, multiplied or reflected. Spikes in a chromatogram can be removed. Peaks can be enhanced. Baselines can be corrected manually or automatically. Two chromatograms can be added, subtracted, divided or appended. With the Gaussian curve approximation small parts of a chromatogram can be integrated. The menubar of **Transform** contains the points:

Modify Arithmetics Baseline Curve Fitting Exit Window Help

4.8.1 Transform - Modify

After clicking ***Modify*** a file select box appears where a chromatogram can be chosen. The chromatogram is shown in the upper part of the window.

The menu bar displays the menu points:

File Calculation View Expand Window Help

The next step, "Fix Calculation Mode", is displayed in the information box.

The following operations are available under ***Calculation***:

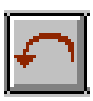
Smoothing a chromatogram.

1. Derivative constructing the 1st derivative.

2. Derivative constructing the 2nd derivative.

Smoothing (digital filtering) and constructing of derivatives are done according to the Savitzky/Golay method. The number of points used in this algorithm are defined as Convoluting Points (5,7,9,11,13,15) under ***Parameter.***

Multiplicate multiplies the mV values of a chromatogram with the factor defined under ***Parameter*** in the box *Multiplication* against *Factor*.



Reverse turns the chromatogram, so that the starting point is now at the end of the time axis.

The ***Clean*** function removes spikes (high mV-values for a very short time period) in the chromatogram. After clicking ***Parameter*** the maximum time period of a spike can be defined against *Max. Time* and the minimum signal height against *Min.Height* in the *Clean* box.

Filter smoothes the chromatogram in a different way as under *Smoothing*.

Enhancement causes a peak enhancement. After clicking ***Parameter*** the frequence can be entered against *Filter Cutoff Frequ.* in the *Filter* box. For this frequence the intensity values in the chromatogram are set to 0 after Fourier-Transformation.

Slice Width Conversion allows the slice width to be changed (only increased).

Selecting the menu point ***Parameter*** displays a dialog box where the parameters for the different calculations can be changed. Exit by clicking OK.

After this a new calculation can be performed by clicking *Calculation* and selecting one of its submenu points.

Parameter to be changed:

Convoluting Points

Number of points which are used as averaging basis, values from 5 - 15, odd numbers.

Multiplication, Factor

This factor is used for multiplicating the chromatogram, values without limit.

Filter - Cutoff Frequ.

All frequences greater than Cutoff. Frequ. , e.g. high frequent noise, is filtered off, thus smoothing the chromatogram.

Enhancement - Slope Width

In this calculation, the difference between adjacent points is enhanced by multiplication and devision, respectively. The parameter Slope Width (in min) determines the width of the neighbourhood of a point. The parameter should be smaller than the peak width.

Clean

When *Autoclean* is clicked for removing spikes, a maximal peak width (in min) must be defined against *Max. Time*. After clicking OK and activating *Calculation, Clean* all peaks smaller than the peak width entered are removed.

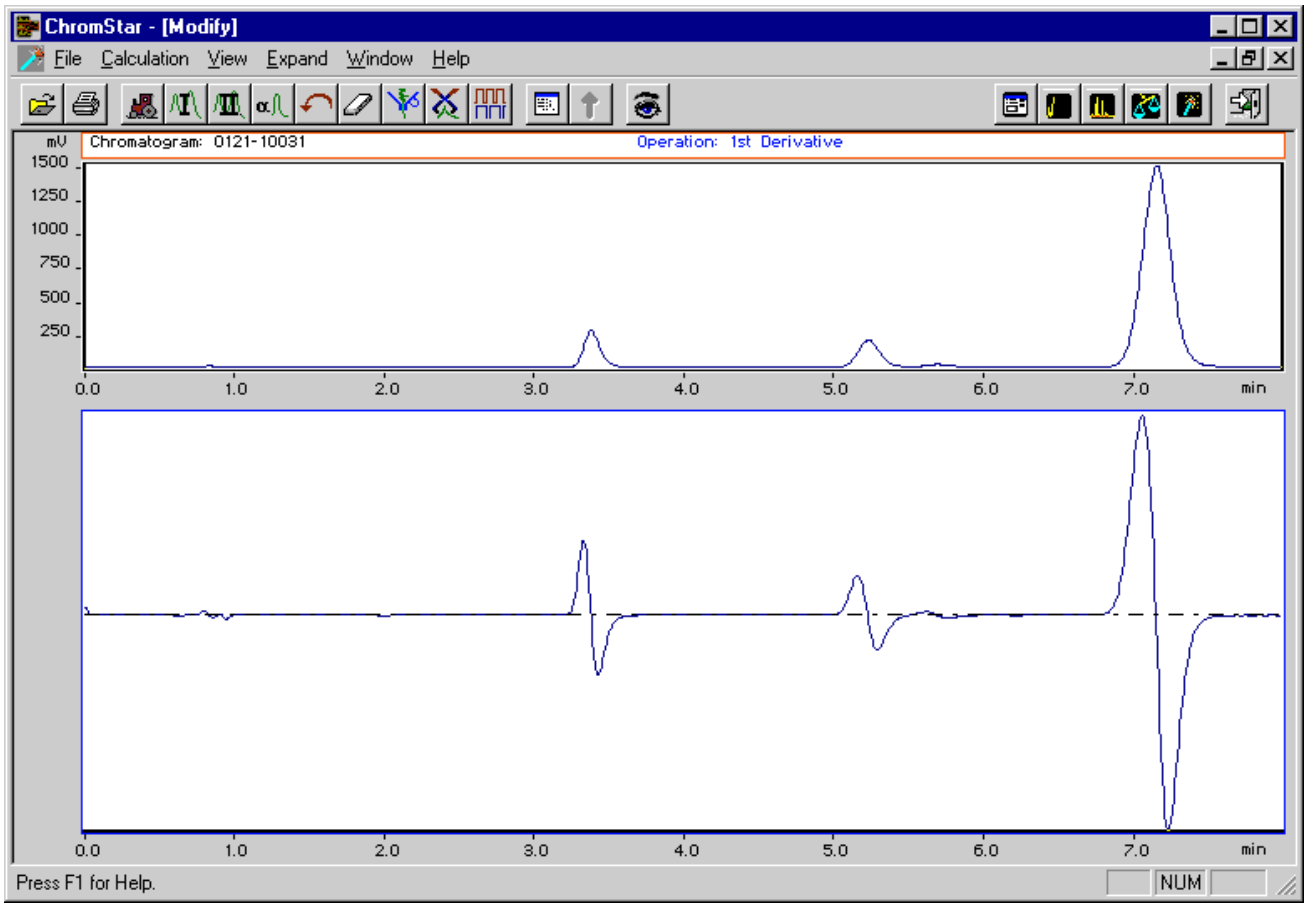
Without *Autoclean* the cursor appears as rubber. After activating *Clean*, a peak is removed by clicking into it. Only integrated peaks can be removed. If more than one peak is to be removed, the result can be moved in the upper part by using *Swap* and then be calculated again.

The resultant chromatogram can be saved via *Save as* and then be reintegrated.

Swap moves the resulting chromatogram from the lower box into the upper one, where it can be calculated again.

The result of the calculation is displayed in the lower window when the calculation is finished.

The calculation method is displayed in the information box.



Enlargement of a section of the upper chromatogram is possible by constructing a rectangle as already described. The enlarged section then appears in the lower window. Another section can be enlarged after clicking a point anywhere in the upper sector. The original picture can be regained by clicking in the upper range with the right mouse button.

File and its submenu offer various file operations

With ***Open...*** a new chromatogram can be selected for evaluation.

With ***Save as...*** the result of a smoothing (file extension .SLI) can be stored. The original chromatogram may not be overwritten. The 1st and 2nd derivatives cannot be stored.

File Information displays a box with entries about: Author, Sample Identifier, Recording date, Changes, Number of datapoints, Runtime, Minimum and maximum mV-value.

The menu point ***Print*** with its options *Dump* and *List* allows the lower window contents to be printed (**21).**

Printer Setup allows the printer settings to be changed.

Copy transfers the presentation to the clipboard.

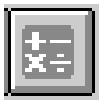
Exit returns you to the **File Transformation** window.

By clicking the menu point ***View, Show Values*** an information box appears with the retention time and detector signal value or the value of the 1st or 2nd derivative depending on the position of the mouse cursor in the chromatogram which is indicated by a perpendicular white line. The box disappears after clicking *View* again.

Expand, By Values... allows an enlarged section of the upper chromatogram to be displayed by value entries for the time axis and for the y-axis (in mV), the corresponding calculation appears in the lower field. *Expand* can also be used before executing a calculation. *Set Default* restores the original values. After clicking OK the original chromatogram appears together with the calculation.

4.8.2 Transform - Arithmetics

This program point allows you to add or subtract two chromatograms, to display the ratio between them, or to append them.

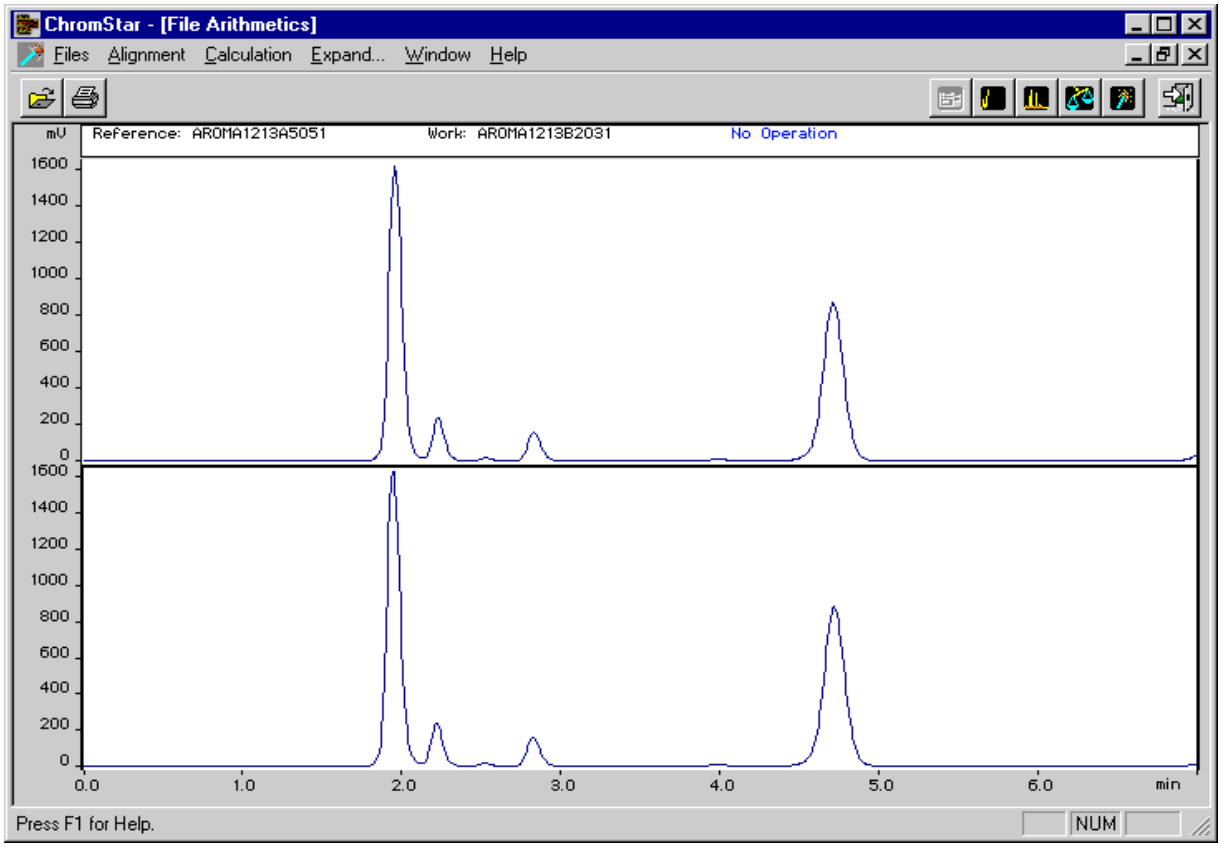


After selecting the menu point ***Arithmetics*** a dialog box appears for selection of the chromatograms to be processed, these are described as the *Reference* and the *Work* chromatogram. When selected, the *Work* chromatogram appears in the upper half of the screen and the *Reference* chromatogram in the lower.

The title bar now carries the text **File Arithmetics** and the menubar contains the points:

Files Alignment Calculation Expand... Window Help

Only chromatograms recorded with the same slice width (cp. 4.1.2) or slice widths which are integer multiples of one another can be used in these calculations. If chromatograms have been selected that do not meet these requirements the message "Slice Width Mismatch" appears. Clicking OK allows new chromatograms to be selected.



The menu points have the following meaning:

Files with its submenu points permits new chromatograms to be selected (***Open***..).

File Information displays a box with entries about: Author, Sample Identifier, Recording date, Changes, Number of datapoints, Runtime, Minimum and maximum mV-value.

Print, ***Portrait*** or ***Landscape*** allows *Work* and *Reference* chromatograms to be printed.

Printer Setup allows the printer settings to be changed.

Copy transfers the chromatograms to the clipboard.

Exit closes the **File Arithmetics** window.

Alignment. In the next work stage, which can be carried out before the calculation, an alignment of the Work chromatogram along the time axis (*Time Alignment*) and an internal normalisation on a data point (*Height Alignment*) for both chromatograms can be effected.

After selecting the point ***Time Alignment*** the positions in the *Reference* and *Work* chromatograms which are to be matched after the alignment must be defined. In the information box the message "Align the chromatograms" appears. This is done as follows:

A time point is defined in the *Work*-chromatogram by clicking the left mouse button. The mouse cursor is moved to the appropriate point of the *Reference* chromatogram which plots a line between both points. This position is confirmed by clicking the left mouse button. This causes the mouse cursor to return to the point defined in the *Work* chromatogram which can be adjusted if required. The alignment is performed after clicking the right mouse button.

Normalisation of the y-axis (***Height Alignment***) is performed as follows:

After clicking the left mouse button in the *Reference* chromatogram a vertical white line appears which can be positioned by moving the mouse. Confirm the position with the right mouse button.

Upon this a factor is calculated so that the intensities of both chromatograms are identical at the marked position. The entire *Work* chromatogram is now multiplied with this factor and displayed again.

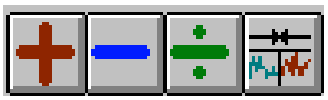
Both before and after the alignment an enlargement can be made in the lower chromatogram. This is always automatically normalized to the highest data point. Clicking the right button in the lower sector restores the original scale. The alignment is not affected.

Expand allows an enlarged section to be displayed by value entries for the time axis. Also here an automatic normalisation to the highest datapoint is performed.

Calculation. After selecting the menu point *Calculation* both original datasets are displayed in the upper half of the screen; the lower half is reserved for displaying the results.

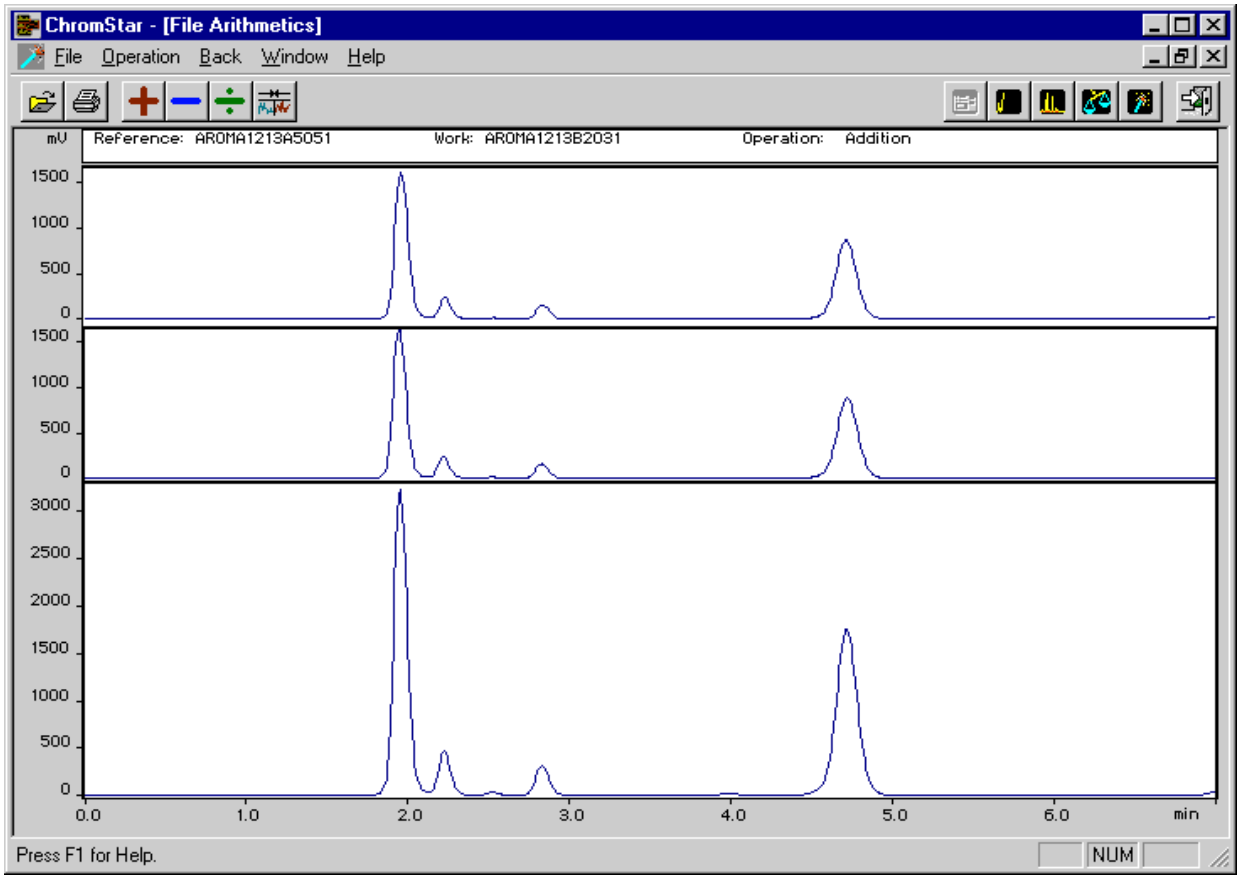
The menu bar now contains the points

File Operation Back Window Help



After selection of one of the points of ***Operation*** (***Addition, Subtraction, Ratio*** or ***Append***) a calculation takes place and its results are displayed in the lower part of the screen.

In the Subtraction procedure the W*ork* chromatogram is subtracted from the *Reference* chromatogram. *Ratio* displays the ratio from *Reference* chromatogram to *Work* chromatogram. The information box shows behind " Operation" which procedure has been used. If the calculation is only to be performed for a section, this must first be enlarged by drawing a rectangle in the lower chromatogram before calculation takes place.



The menu point ***File*** now contains the submenu points:

Save As... for storing the results. A dialog box enables you to define the name of the result chromatogram which receives the file extension .SLI. The original chromatogram cannot be overwritten.

The result of the ratio display cannot be stored.

Print with its submenu ***Portrait*** and ***Landscape*** prints out the *Reference* and *Work* chromatograms and the result (**22**).

Printer Setup allows the printer settings to be changed.

Copy transfers the chromatograms and the result to the clipboard.

Exit returns you to the **File Transformation** window.

Back returns to the display of the original chromatogram. Here other *Work* and *Reference* chromatograms can be selected with *Files* and *Open*....

4.8.3 Transform - Baseline

The menu point ***Baseline*** with the points ***Automatic*** and ***Manual*** allows you to correct the detector baseline of a chromatogram either automatically or manually.

4.8.3.1 Transform - Baseline - Automatic

After clicking ***Baseline*** and ***Automatic*** and selecting the chromatogram, this appears in the upper third of the screen. The title bar reads: **Automatic Baseline Correction.** The menu bar contains the points:

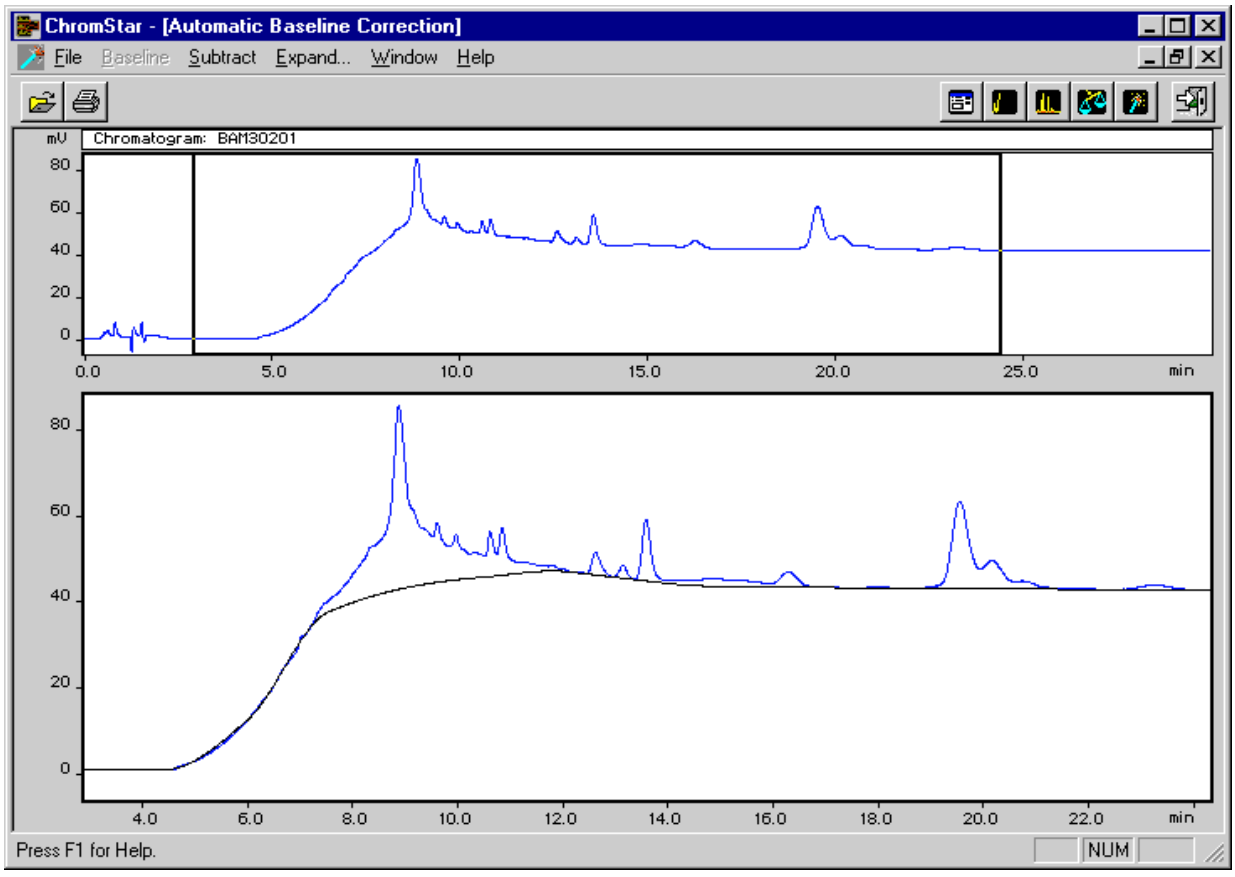
File Baseline Subtract Expand... Window Help

The information box reads "Select rectangle to zoom".

Expand... allows an enlarged section in the upper window to be created by value entries.

To carry out the baseline correction first construct a rectangle. The created enlargement appears in the lower part of the display.

After clicking ***Baseline*** a corrected baseline is calculated. This is shown as a red line in the lower display.



With ***Subtract*** the area beneath the corrected baseline is subtracted from the chromatogram.

File and its submenu offer various file operations.

Open... returns you to selection of a further chromatogram for automatic baseline correction.

Save As... stores the result. Ensure that you do not overwrite the original chromatogram when doing this!

File Information displays a box with entries about: Author, Sample Identifier, Recording date, Changes, Number of datapoints, Runtime, Minimum and maximum mV-value.

Print with its submenu points ***Portrait, Landscape*** and ***Chart Speed*** prints out the lower section of the screen.

Printer Setup allows the printer settings to be changed.

Copy transfers the result to the clipboard.

Exit returns you to the **File Transformation** window.

According to the status of the operation the menu points ***Baseline*** and ***Subtract*** and the submenu point of ***File, Save As...***, are not accessible and appear in light grey.

4.8.3.2 Transform - Baseline - Manual

Manual correction of the baseline is performed by clicking ***Baseline*** and ***Manual*** and selecting a chromatogram.

The title bar reads: **Manual Baseline Correction**.

The menu bar contains the points:

File Order Expand... Window Help

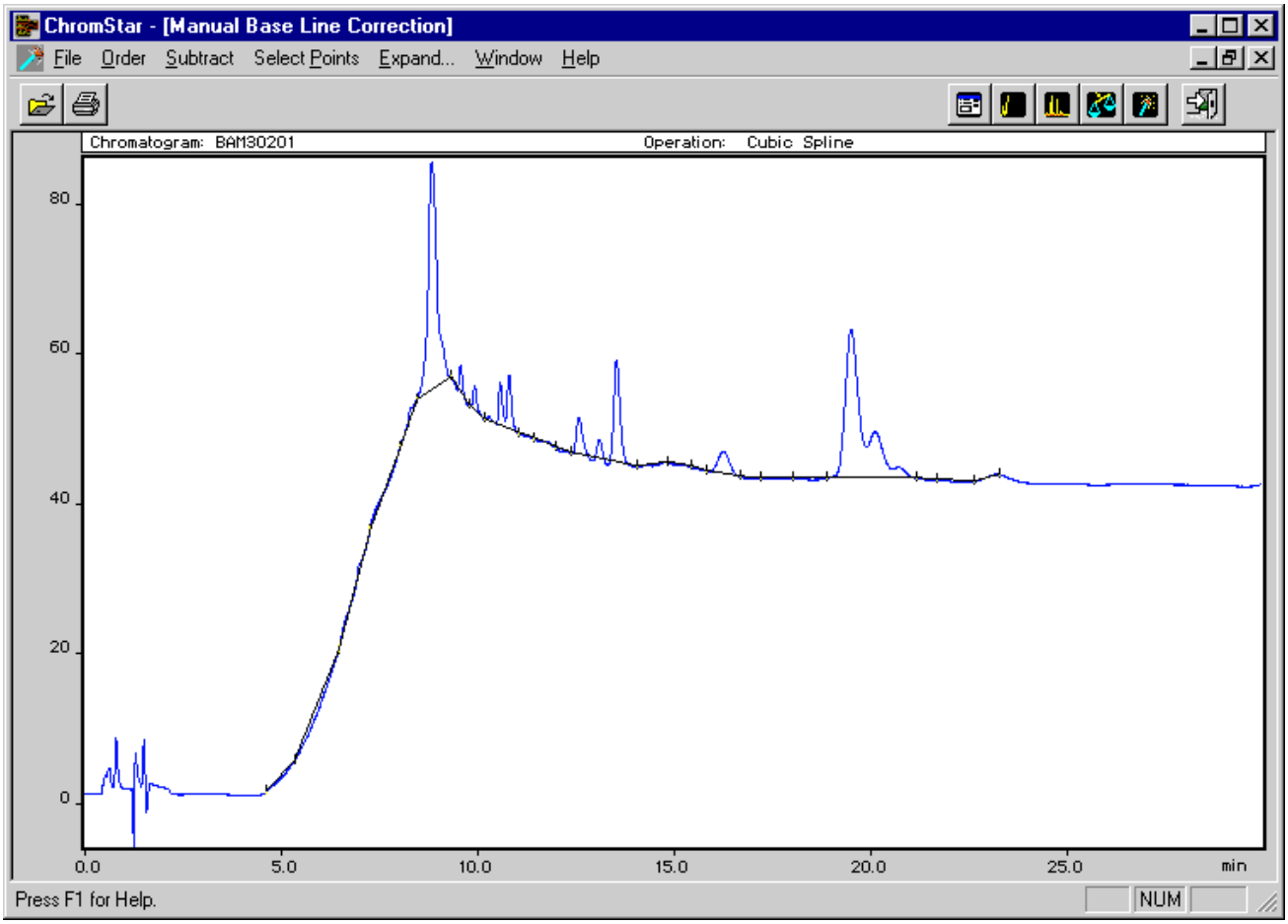
Here too an enlargement must first be constructed. After this select a maximum of 25 points in the lower screen part by clicking with the left mouse button to points through which the new baseline has to be plotted. A point can be deleted by clicking with the right mouse button.


The menu point ***File*** and ***Open*** returns you to chromatogram selection. ***Exit*** returns you to **File Transformation**.

File Information displays a box with entries about: Author, Sample Identifier, Recording date, Changes, Number of datapoints, Runtime, Minimum and maximum mV-value.

Expand allows an enlarged section of the upper part to be displayed by value entries for the time and mV-axis.

Calculation of the approximation function for the new baseline is effected in the menu point ***Order*** which offers the possibility of conducting an approximation of the first through the fifth order and of a ***cubic spline*** approximation. The options offered are determined by the number of baseline points which have previously been defined. If only two points in the chromatogram have been defined it is only possible to plot a straight line between them which is later subtracted from the chromatogram. A minimum of four points are necessary for a quadratic approximation and five for a cubic approximation. For approximations of the 1st to 5th order approximation functions are calculated based on the method of least squares. The points selected need not necessarily lie on the approximation function. For the ***cubic spline*** approximation a function is calculated in which all points are on this approximation function. After the desired approximation has been clicked the complete chromatogram with the calculated baseline appears on the screen.



The menu bar contains the points:

File Order Subtract Select points Expand... Window Help

Order allows the order of the approximation function to be changed.

Subtract deducts the calculated baseline from the complete chromatogram. The menu point ***Order*** is now no longer accessible.

The menu point ***File*** allows various options again.

Open allows you to select a new chromatogram.

The result of the baseline correction can be stored with ***Save as...*** and the definition of a file name (extension .SLI). If Subtract has not yet been clicked the message "No Result File" appears. The original chromatogram cannot be overwritten.

File Information displays a box with entries about: Author, Sample Identifier, Recording date, Changes, Number of datapoints, Runtime, Minimum and maximum mV-value.

Print with its submenu ***Portrait, Landscape*** and *Chart Speed* prints out the chromatogram.

Printer Setup allows the printer settings to be changed.

Copy transfers the screen contents to the clipboard.

Exit returns you to the **File Transformation** window.

Select-points allows the entry of new points for plotting of the baseline. To do this you must again create an enlargement in the lower section by constructing a rectangle.

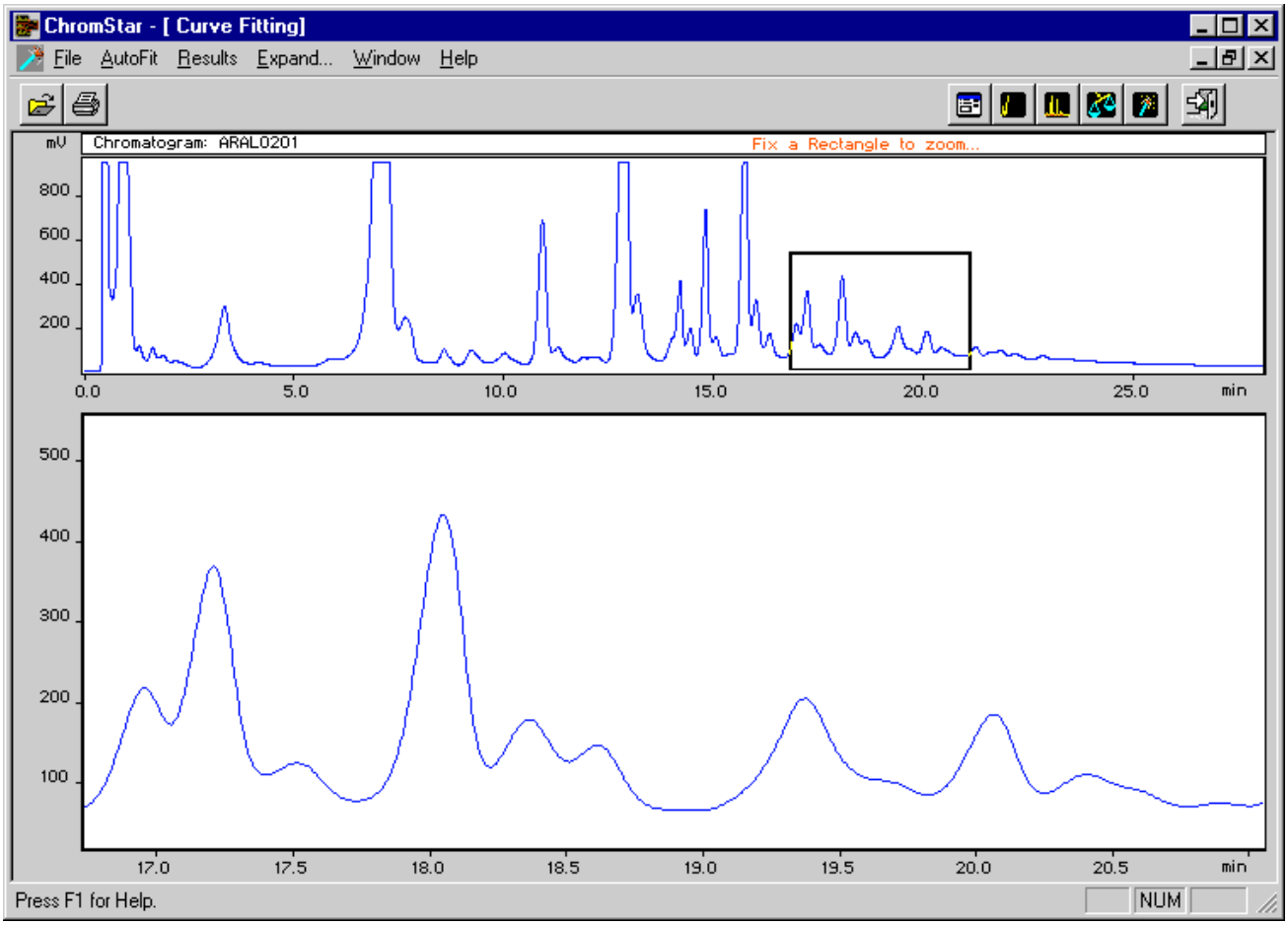
4.8.4 Transform - Curve Fitting

The menu point ***Curve Fitting*** allows the reintegration of an enlarged sector of the chromatogram by means of an approximation of the peaks with a Gaussian function.

To carry out this reintegration, first select a chromatogram and define the part to be examined by constructing a rectangle.

The window title bar reads **Curve Fitting**. The menu bar contains the points:

File Autofit Results Expand... Window Help



After selecting the point ***Autofit***, three approximation algorithms are offered:

Peaks: Single - Determination of the peaks by means of a Gaussian function

Peaks: Double - Determination of the peaks with a Gaussian function on both peak flanks

Peaks: Rational - Determination of the peaks with a fractional-rational function of the fourth order.

After clicking one of these three evaluation options, a window appears in which the number of peaks to be calculated can be entered. The default number of peaks is set to 4.

OK starts the calculation. The number of peaks calculated can be set between 1 and 20 whereby the calculation time will be considerably longer the larger the number of peaks to be evaluated. Depending on the sector, the number of peaks which can be evaluated is limited. The peaks are calculated in descending order of their heights.

The calculation can be interrupted by Cancel. After the calculation has been carried out the result is displayed on the screen.

File and its submenu offer various file operations:

Open... allows a new chromatogram to be selected.

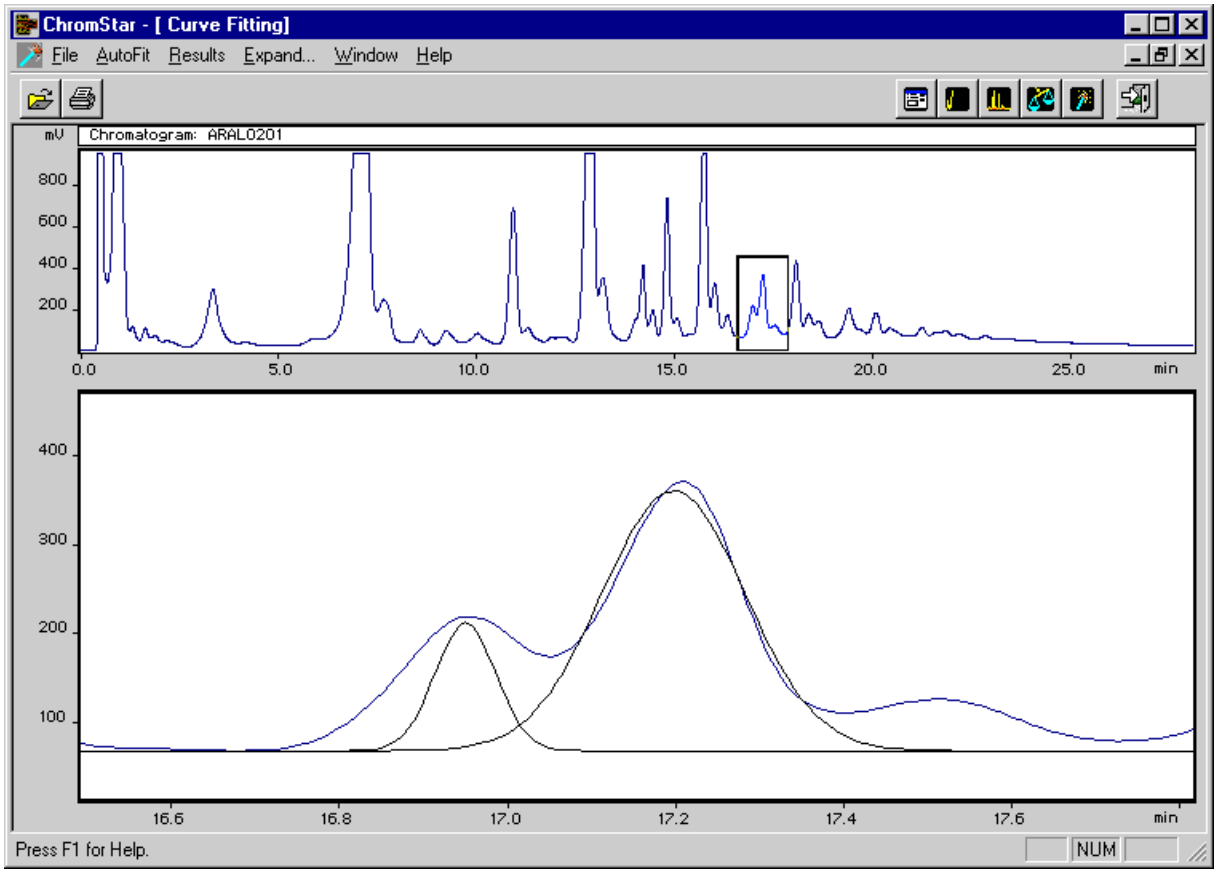
File Information displays a box with entries about: Author, Sample Identifier, Recording date, Changes, Number of datapoints, Runtime, minimum and maximum mV-value.

Print allows you to print-out the chromatogram sector with its calculated peaks analysis or the results list either in landscape (***Landscape***) or in portrait (***Portrait*****)** format or with an individually selected chart speed (***Chart Speed***).

Printer Setup allows the printer settings to be changed.

Copy transfers the sector to the clipboard.

Exit returns you to the **File Transformation** window.



The menu point ***Results*** offers the possibility of checking the result.

Show Fit redraws the individual peaks so that, after using the following submenu points, the original graph is restored without having to repeat the calculation.

Sum shows the sum of the individual peaks. This appears as a white line in the chromatogram and shows how the approximation corresponds to the chromatogram.

Difference subtracts the sum of the peaks from the chromatogram. This difference also appears as a white line and shows the degree to which the baselines differ.

Report displays the results list of the chromatogram sector with peak numbers, retention times, peak areas, area percentage and peak heights. The peak areas and heights calculated can be compared with values obtained by automatic integration.

Peak numbers are only allocated for the sector of the chromatogram selected. Because of the possible long calculation time, always a small sector only should be selected for peak analysis by *Curve fitting*.

Expand... allows an enlarged section of the upper part to be displayed by value entries for the time axis and for the y- axis.

5. Chromatography with ChromStar

5.1 Data Acquisition

5.1.1 Recording a Chromatogram

In this section the user will be instructed how to quickly record a chromatogram without reading the whole manual first. Shown here is how to set the parameters for solving the next task:

A chromatogram has to be recorded of a test mixture using an isocratic mobile phase of 60% methanol (in solvent container A) and 40% water (in solvent container B) at a flow rate of 1 ml/min, and a run time of 12 min with an RP 18 column and a UV detector at 254 nm.

First switch on the computer, the monitor, the printer, the detector and the pump. The detector signal is connected to channel 1 of the A/D converter board. The pump is controlled via the RS232 - Comm. Port 1.

The operating system is loaded, in case of automatic ChromStar loading the title page of ChromStar appears.

The next step is to create the files necessary for the analysis:

Method Data Handling LC-Procedure

If a pump is used which is not controlled via ChromStar it is not necessary to create an LC-Procedure file. All entries concerning the solvent can be skipped.

For that purpose click ***Edit-Files*** in the menu bar of the **ChromStar** application.

Edit-Files Analysis Reprocess Transform Window Help

Now the **Edit-Files** Window appears on the screen with the menu bar:

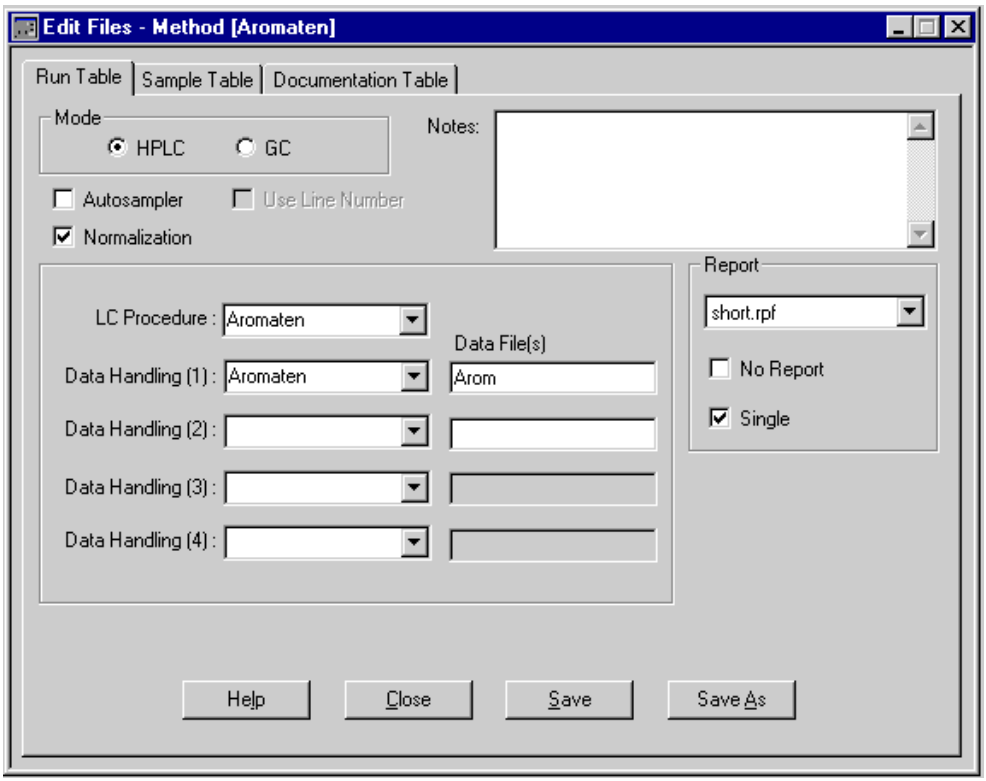
Chrom.Files Options Exit Edit Files Window Help

After selecting ***Chrom.Files*** and the submenu point ***Method*** a dialog box appears with, on the left, a list with already existing Method-Files and an entry field, and on the right, a field for changing the subdirectory. Because creating a new method is explained here, first a new name (max. 23 characters: .,\ and umlauts are not allowed) is entered, in this example AROMATEN, the window is left by clicking OK.

Following upon this the method file appears.

To obtain a print-out after recording the chromatogram Report is selected and the report print template SHORT.RPF is chosen. It is not necessary to change all other parameters.

It is of course possible to enter a name and other appropriate information in the *Notes* field.

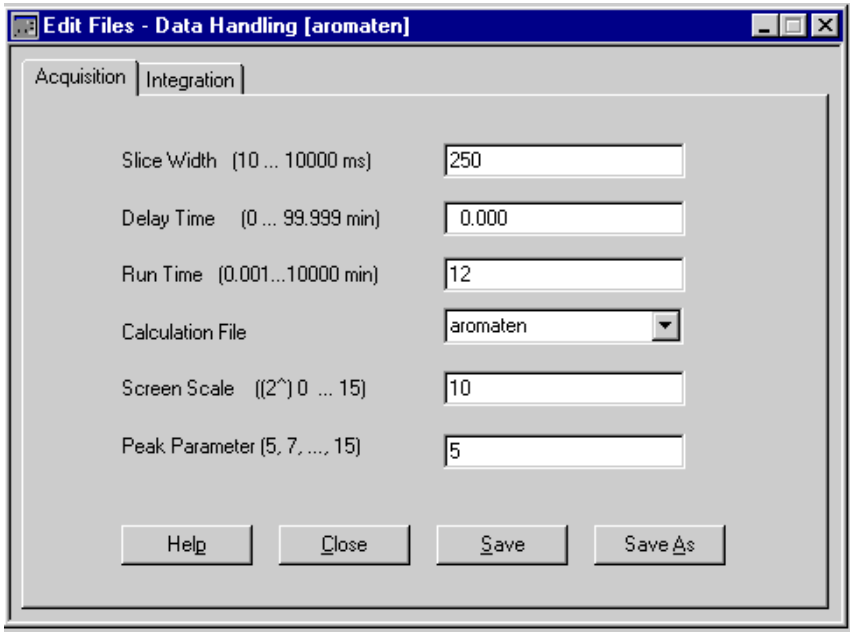


The necessary Data Handling and LC Procedure files automatically receive the default name AROMATEN.

The chromatograms (Data Files) are named with the first characters AROM.

The file is closed with the screen key ***Close***. In a small dialog box the message appears "AROMATEN is a new file" and the question "Save it?", which is answered with Yes.

Again the **Edit Files** Window appears in which a data-handling-file can be created after clicking ***Chrom.Files*** and ***Data Handling***. In this case, where it is important to build an analysis with a minimum number of steps, the data-handling-file must get the the same name as the method-file. The name AROMATEN is entered in the entry line and confirmed with OK.



Now the acquisition page of the data handling file AROMATEN appears on the screen. The value applied up till now of the parameter Slice Width is shown on a black ground and can be overwritten. Here 250 should be entered (this is an appropriate value for a minimal half height peak width of 5 sec).

After this the desired run time, in this case 12 minutes, has to be entered. This is done by striking the TAB key twice to reach the parameter Run Time and entering the value 12.

The name AROMATEN is automatically entered against Calculation File. This file does not have to exist.

Now the file is saved as described for the method file.

If your HPLC instrument is not controlled by ChromStar, or instead of an HPLC separation a gas chromatogram is to be recorded, then skip the following section.

After clicking ***Chrom.Files*** and its point ***LC-Procedure*** enter here as well the file name AROMATEN.

Now the field as shown in figure 90 appears (but without entries), in which the solvent composition to be pumped must be defined.

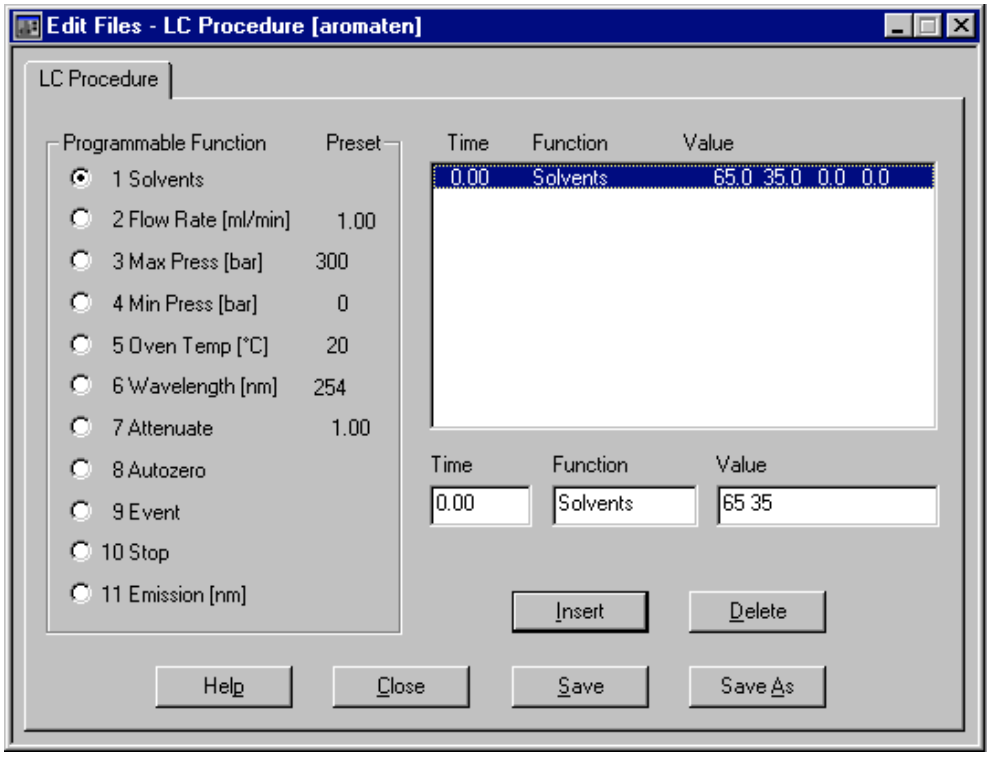
The cursor is blinking in the Time box. With the key sequence:

TAB (defining the time)

TAB (selects the function "Solvent")

60 space 40 (solvent A=60% and B=40%)

the eluent composition is defined. On clicking Insert the line is transferred into the table above. No further entries are necessary in the LC-Procedure-file.

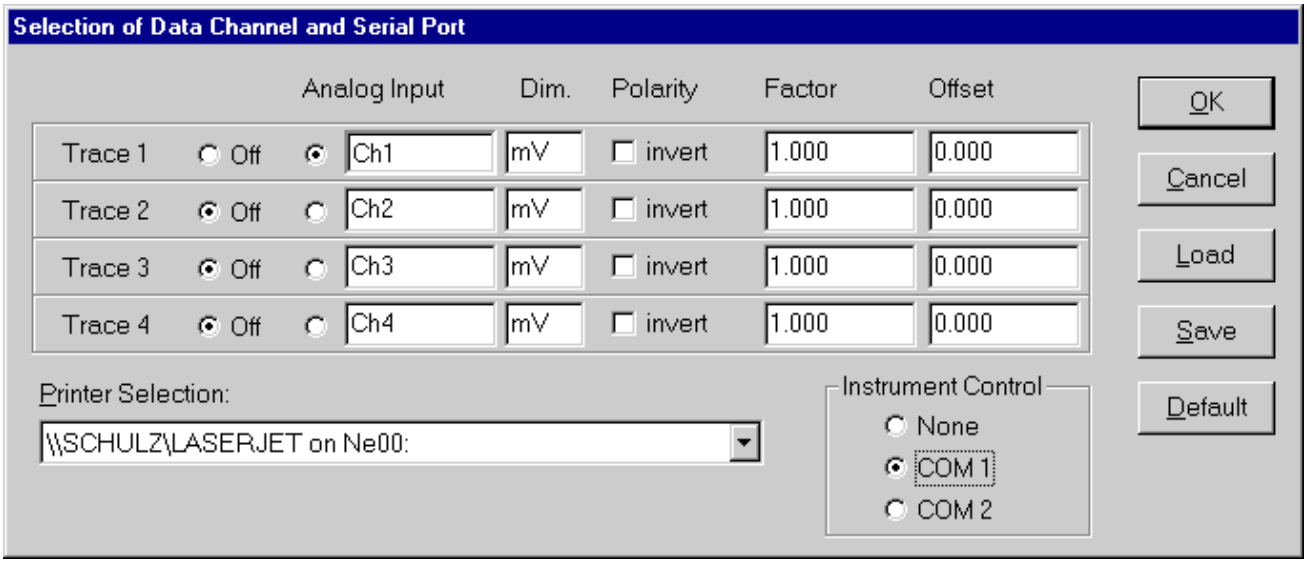


The file is saved by clicking File and Close and answering Yes against the question "AROMATEN.PRO is a new file. Save it?".

The **Edit Files** Window is left with ***Exit Edit Files***.

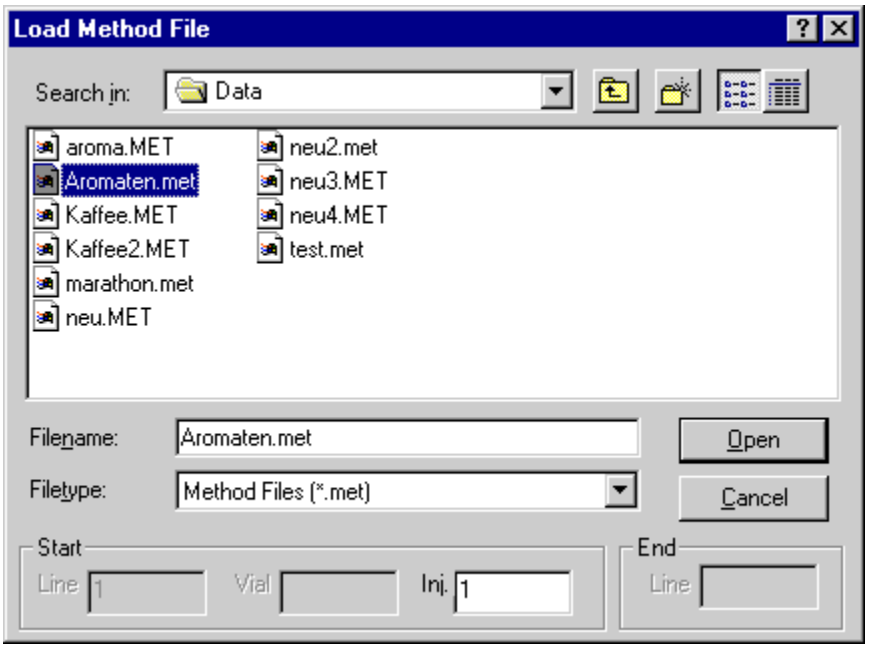
All the necessary files are now created, and the method can be started as described in the following section.

Therefore click the menu point ***Analysis-Chromatogram*** in the **ChromStar** window. To choose the data acquisition channel and, if available, an HPLC instrument click click Trace 1 and in case of instrument control COM 1. Against Printer Selection the selected printer is shown. The box is left with OK.



In the lower part of the **Draw Baseline** window a line appears with details about the flow rate and eluent composition. The detector signal is displayed in the black area and the value of the detector signal is shown in the right top corner.

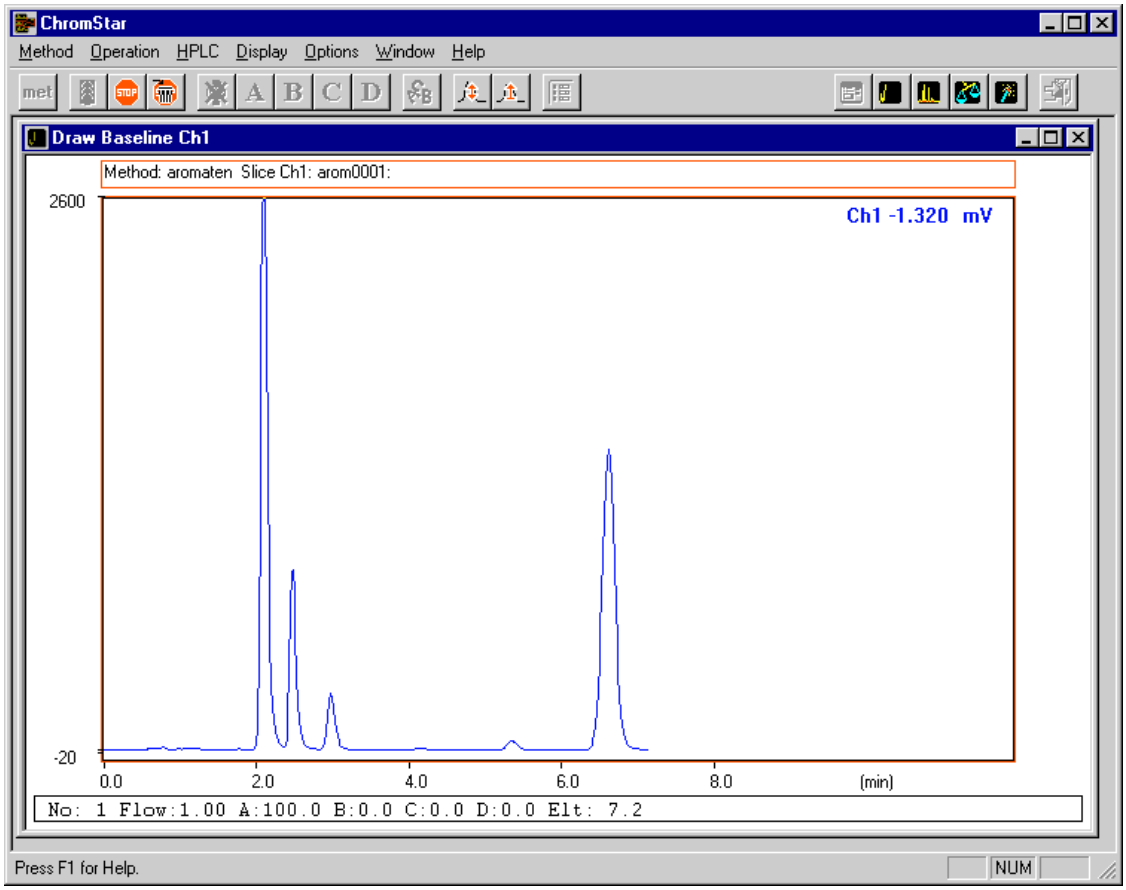
The previously created method AROMATEN can now be recalled by clicking ***Method..., Load*** and selecting AROMATEN by clicking it in the directory with all method files which now appears. Leave this box with OK.



In the line above the black area the message "Method: AROMATEN" appears. This shows that the method is activated, in the line below a 1 appears against No, i.e. the next injection receives number 1. When a controlled LC-instrument is used the pump starts to pump the eluent composition as specified in the LC procedure file AROMATEN, in this case 60 % methanol and 40% water.

Wait until the column is equilibrated and the detector signal and the pressure have reached stable values, in other words that the chromatograph is ready to inject.

The sample is brought into the injector, then turn the injector to the inject position and  at the same time press the function key F1. The running chromatogram appears now in the **Draw Baseline** window with a time axis according to the Run Time selected. Above this next to the name of the method appears the name of the chromatogram being recorded in channel one, here AROM0001. In the line with status information the time elapsed is displayed against Elt. In order to have an  optimum screen display of the data, the display was changed in this case with F11 (Select Screen Scale), clicking By Values (low value = -10, high value = 800) and OK.

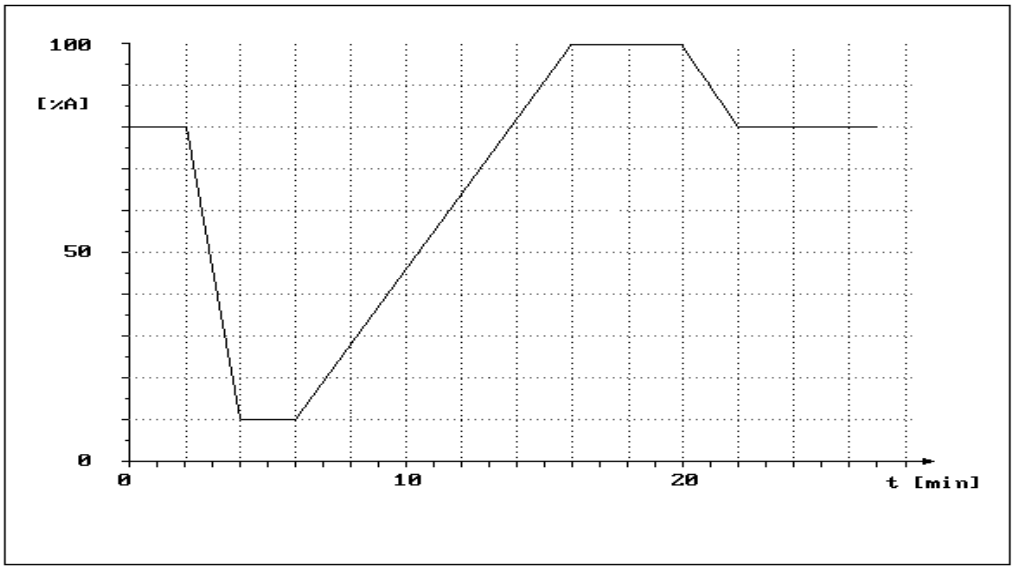


 After 9 minutes the data acqisition is stopped. After the calculation (Calculation in Process), the data are sent to the printer and the chromatogram is printed out on one DIN A4-page in portrait format, below it the results table (**6**) appears. The data are saved under the name AROM0001.SLI. In the Report-File, which is created at the same time, the results of the integration (retention times, peak areas and heights) are stored together with all parameters used for recording and evaluation.

 The recording of the chromatogram is now finished and the pump stopped with the function key F9.

5.1.2 Programming a Gradient

To program a gradient with two solvents and the following shape:



and the time table

0 min		A	80%	B	20%
2 min		A	80%	B	20%
4 min		A	10%	B	90%
6 min		A	10%	B	90%
16 min	A	100%	B	0%	
20 min	A	100%	B	0%	
22 min	A	80%	B	20%	
25 min	A	80%	B	20%	


an LC-Procedure file is created by first clicking ***Edit Files*** followed by ***Chrom.Files*** and ***LC Procedure***. A desired name, in this example GRADIENT, is entered in the entry box above the directory. After clicking OK the entry page of an empty LC procedure file appears. The cursor is blinking in the entry box Time to show that entries can be made. The following entries are necessary for the gradient described above:

TAB TAB TAB 8 0 space 2 0

Then Insert is clicked or the Return-key (Enter) is pressed. The line appears on a black background in the table above. The box under Time also appears on a black background to show that further entries can be made. Now 2 is entered and Insert is clicked.

To complete programming this example the following entries still must be made:

4 TAB TAB 1 0 space 9 0 Insert

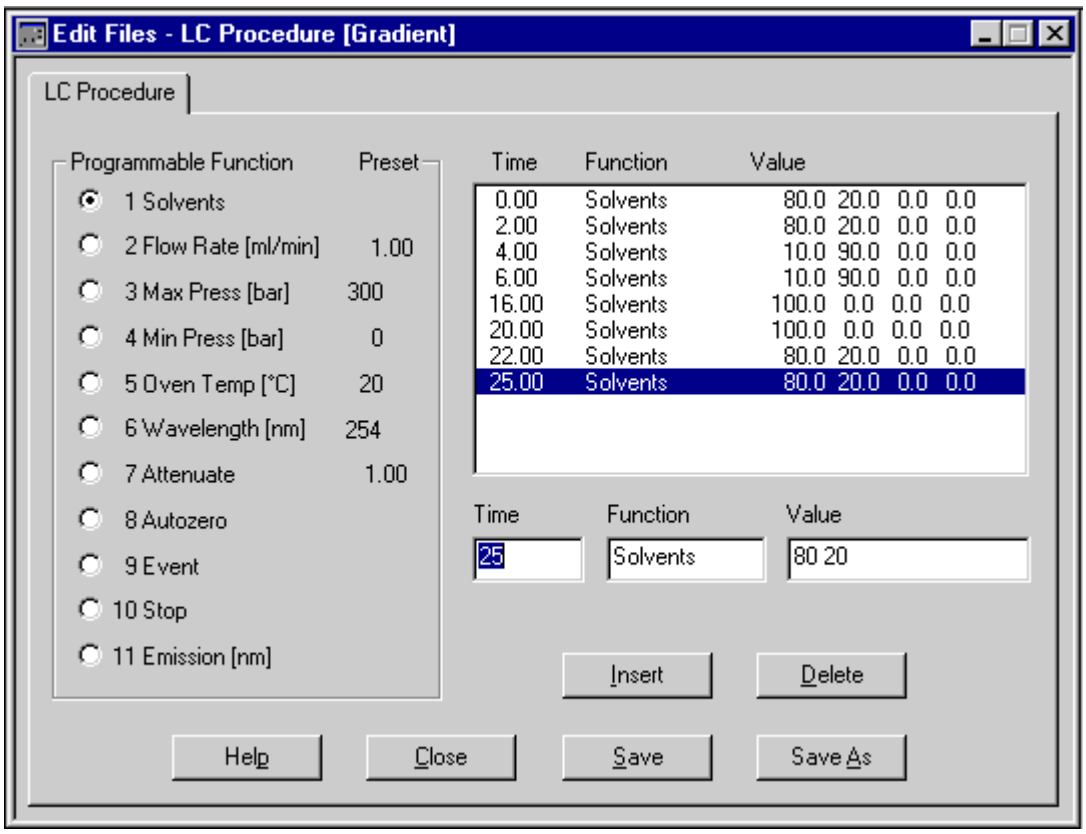
6 Insert

1 6 TAB TAB 1 0 0 Insert

2 0 Insert

2 2 TAB TAB 8 0 space 2 0 Insert

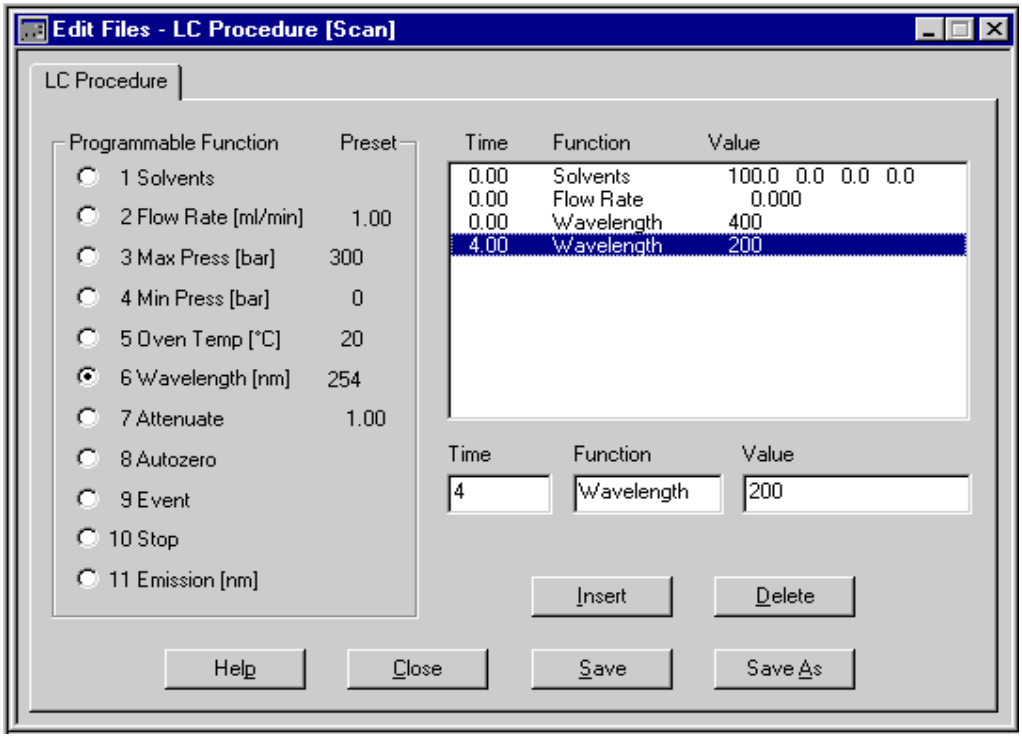
2 5 Insert



The LC procedure file GRADIENT can be stored by clicking ***File*** and ***Close*** or the screen key and then clicking the box "Yes" against the question "Save it?". Now the file can be used in a chromatographic run and for this purpose it can be entered in a Method-File.

5.1.3 Recording a UV spectrum

Using a UV detector with variable wavelength controlled by ChromStar through an interface, a UV spectrum can be recorded when the pump is stopped. For a UV spectrum in the wavelength range of 200 - 400 nm for example the LC procedure file in this case named SCAN will be created.



The entry Flow Rate = 0 causes the wavelength to be scanned in a continuous linear way (wavelength scan) and not in steps (at times 0 min and 4 min). As the absorption in the short wavelength range is usually higher than in the long wavelength range, it is recommended to scan from long to short wavelengths, and also to adjust the detector signal to zero before commencing the wavelength scan, in order to be able to follow the spectrum on the screen. If a measurement is to be made with wavelengths greater than 400 nm it is necessary to install the second order filter.

To carry out the measurement a method with a normal LC procedure and data handling file is started. When the peak maximum of the compound to be investigated appears in the chromatogram, i. e. when the highest amount of the compound is in the detector cell, the pump is stopped with F9 and the method is aborted via F3. The method SCAN, with the LC procedure SCAN and a data handling file SCAN containing a delay time as long as the run time, is started by clicking the submenu point *Method* in the **Analysis** window, entering the file name and pressing F1. The long delay time in the data handling file prevents a senseless evaluation. The same can be achieved by using the parameter Integrate Inhibit during the run time in the data handling file SCAN.

5.1.4 Operating an Autosampler

5.1.4.1 Installing the Autosampler

The autosampler interface is plugged into the backside of the autosampler. In case of using a controllable pump, the RS 232 input on the interface is connected with a nine-pole cable to the RS 232 output of the pump; this in turn is linked to the RS 232 interface of the computer. Alternatively, the autosampler is linked directly with the computer. The instrument number (device address) is set with a screwdriver at the orange screw of the autosampler interface. The small notch indicates the selected instrument number. This must be different to that of the pump.

Under [Configuration] the CHRST32.INI file must contain the entry:

LCn=LC:m;AS:k

in which n is the number of the computer's RS 232 output, m the instrument number selected at the pump and k the instrument number of the autosampler.

The same entries can be made in the ChromStar Configuration window which can be found in the system configuration window. steuerung machen. After changes in the configuration or in the INI-file ChromStar must be started again (cp. fig. p. 5-12).

Switch the pump and the autosampler on one after the other. Depending of the model the autosampler executes a test routine, this can take approximately 20 seconds. Wait until this is finished. **Attention!** The instruments must be in serial mode.

After this the menu point ***Options, Select Channels...*** can be clicked in the ChromStar **Analysis** window.

In the selection box the data recording channel is clicked as well as COM 1 or COM 2, according to the RS 232 interface being used, even if no pump is being used.

5.1.4.2 Programming the Autosampler

Entries concerning the position of the vials and the order in which they are sampled are made in the Method-File. Click the menu point ***Chrom.Files*** and ***Method*** in the **Edit Files** window and create a method in which the Autosampler parameter is selected on the first page (Run Table). In the field down right all entries concerning LC-Procedure and Data-Handling-File appear in grey i.e. they are no longer accessible. Against Data File Name 4 characters can be entered, these are the first characters of the chromatogram name. The following 4 characters are determined by the vial number (from 0 to 999) and the injection number (hexadecimal entry from 1 to F). Section 4.1.1.2 describes how to generate an autosampler table.

The vial number entered against Vial in the first line of the autosampler table is the start number of operations.

A vial entry against the parameter To is the last vial number of the processing sequence. All vials between start vial and the vial under To are processed with the

same conditions. Missing vials generate an error message (cp. sect. 5.1.4.4), the processing sequence is continued.

Depending on the type of autosampler the vial number entered against Vial is the start vial, all following vials are processed in a continuous sequence in exactly the same way as this first one.

A calibration run is defined with S against Type (one-point-calibration). In the Data-Handling-File to be used the name of an existing Calculation-File must be defined, this contains the retention times and standard amounts of the peaks to be calculated.

If more than one injection is made from a vial which is declared with Type = S as standard, the average of their response factors will be entered into the calculation file (defined in the data handling file) against K1 in the regression table.

In order to perform a multi-level-calibration the series of vials with different standard solutions is defined in the autosampler table in **one** line which is marked with S against Type. In the Sample Table the number of standard solution in a vial is entered under Conc.Level. The Calculation-File, of which the name is defined in the Data-Handling-File, contains the corresponding level entries and in the peak-table the amounts in the standard solutions. The Multi-Level-Calibration is performed automatically after recording the chromatograms and the coefficients of the approximation functions (in case of more peaks) are entered in the regression table. It is also possible, with this type of calibration, to perform repeated injections of the standard solutions.

Depending on the type of autosampler an empty position must be left in the sample-tray after the vial(s) with the standard solution, in order to avoid the following vials to be treated as calibrations. The further processing is entered in a new line in the Autosampler-Table.

Depending on the type of autosampler the Run Time in the method file must be at least 2 minutes.

If the Run Time of the data handling file is greater than the Run Time of the method file, the chromatogram will be terminated after the Run Time defined in the method file.

If the Run Time of the data handling file is less than the Run Time of the method file, the chromatogram will be terminated after the Run Time defined in the data handling file but the analysis will continue for the Run Time defined in the method file. Only at the end of this a next injection is made.

It is essential that all data handling files and, if required, LC-procedure and calculation-files, to be used in the autosampler-table are existing.

5.1.4.3 Carrying out an Analysis with the Autosampler

Select the menu point ***Method..., Load*** in the **Analysis** window and recall a method (with autosampler marked). The method must be transferred to the autosampler, this takes about 15 seconds time, you have to wait until this is finished. If the pump is not already pumping, the end of the transfer time can be recognised by the fact that the pump starts to pump the solvent composition of the first procedure.

The analysis sequence can be started with ***Operation, Start*** or the Start button as soon as the column is conditioned. Depending on the type, the autosampler either picks out the first vial programmed and brings it out for sampling or it moves the sample tray into the corresponding position. Then the injection follows and the chromatogram appears on the screen. Before the next injection takes place, the evaluation and a part of the print-out must be awaited. The display against Vial shows from which vial the injection was made; No. shows the injection number, this is consecutively numbered if repeated injections from one vial are made. The chromatogram name also shows from which vial the injection has been made.

Depending on the type of autosampler any vials in the sample-tray, following after the defined vials, will be processed until no more vials are found. When using the pump you will notice that the method has not been correctly finished by the fact that the pump continues to pump solvent.

The next line of the method is transferred. The number of the next vial programmed appears on the screen against Vial. The injection takes place shortly after this.

After the injection of the last vial of a series - provided that no further entries have been made in the method - a print-out is made. The pump does not stop.

Start may only be pressed again after reactivating the method. In any case the entry against Data File in ***Edit Files, Chrom.Files, Method*** must be changed in order to prevent overwriting of the already existing chromatograms. Before pressing the Start key wait until the pressure and detector signals have re-stabilised.

When aborting an analysis by ***Abort*** or stopping the analysis by ***Stop*** the autosampler aborts the run and the needle is moved to the home position. The method is no longer active. After *Stop* the recorded chromatogram is stored and printed. The method must be activated again before the next start can be carried out. By entering a line number of the autosampler table, a vial and injection number the method can be continued.

Stop Int. When using an autosampler causes the chromatogram to be stopped and saved bewirkt, only after the autosampler run time is elapsed the next injection will be carried out.

Continue can be used during an autosampler run to stop a chromatogram before the programmed end of the run time. The question "Stop data acquisition" is answered by Yes, the chromatogram is stored and the autosampler carries out the next injection.

When ***Unlimited Runtime*** was switched on during the autosampler run, the chromatogramm will be stopped and saved by ***Continue*** or clicking ***Unlimited Runtime*** again, the next injection will be carried out.

5.1.4.4 Trouble shooting and error messages

If the system hangs up for any reason, the best thing to do is to switch off the pump and autosampler than reset your computer and/or start WINDOWS once again. Then start ChromStar again, switch on the devices and activate the RS 232 interface again via ***Analysis, Chromatogram*** and ***Options, Select Channels...***.

When a pump and the autosampler is connected to the computer via RS 232 but the autosampler is not in use, the autosampler must be disabled in the chrst32.ini file in the section [Configuration] by the entry:

Disableautosampcom1=Yes

or

Disableautosampcom2=Yes

After this change ChromStar must be started again.

When the autosampler is not in use and not disabled it must be switched on and set to serial mode. If that is not the case no method can be chosen, the menu procedure *Method...* appears in light grey. In the *Selection* box the Comm port can not be chosen.

Whether an autosampler is used in a method file or not can be seen in the *Select Method File* box. If an autosampler is used entries can be made in the Start box against *Line* and *Inj* and in the End box against *Line*. If a method without autosampler is used only entries in the Start box against *Inj*. are possible.

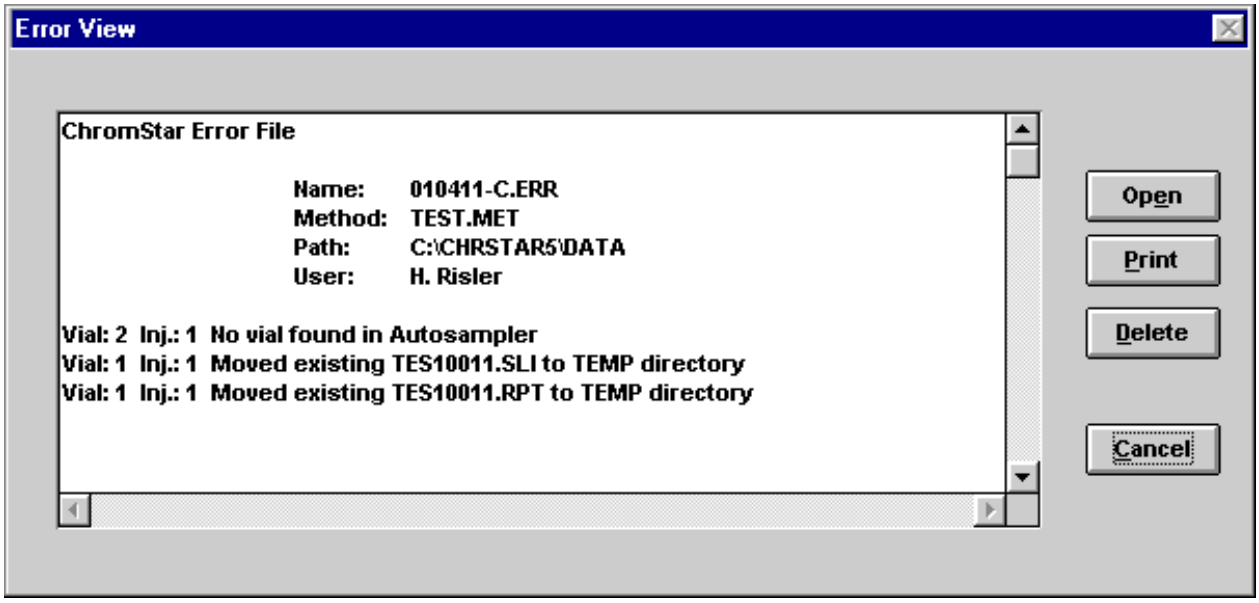
When in an autosampler table a vial is programmed in more than one line chromatograms with the same name are created. To avoid overwriting of existing chromatograms these are moved into a sub-directory of the data directory with the name TEMP and the number (e.g.001) of the line in which the vial is used first.

In the information line the message *ERROR* appears. The full error message can be seen in the *Error View* box accessable via ***Error*** .

When a vial programmed in the autosampler table can not be found in the sample table of the autosampler the message *ERROR* appears in the information line and in the *Error View* box .

The error messages are saved in an .ERR-file. The file can be printed via *Print*. Use *Open* to open other error files. *Delete* deletes an error file. *Cancel* closes the *Error View*-box.

When opening a method the *Error View* box is automatically emptied.



5.1.5 Short instruction guide

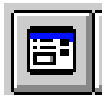
The short instruction guide describes how ChromStar acquires data, records chromatograms, calibrates and evaluates.

ChromStar and the A/D converter board must be installed in the computer. A printer must be connected to the computer. The detector signal must be connected to sockets 9 and 10 of the A/D converter board. The HPLC instruments must be ready. The shortest possible entries and the filename TEST are used in the following description.

Recording Chromatograms

Program Start

Double click on the program icon
(or Start bottom, to the left, Program, ChromStar, click into ChromStar)
in the **ChromStar User-Login** box: press OK or enter a name and press OK.



Creating a Method file

Press Edit Files button (or ***Edit Files*** menu point),
Press met button (or ***Chrom.Files, Method*** menu) , in the **Open Method File** against *Filename*: overwrite UNTITLED by TEST, press ***Open***, deselect *No Report*, select *Single*, click downwards arrow after *Report*, choose report print template SHORT.RPF, press ***Close*** key, question: Save it? Yes

Creating a Data-Handling file

Press int button (or ***Chrom.Files, Data Handling*** menu point), in the **Open Data Handling File** box against *Filename*: overwrite UNTITLED by TEST, press ***Open***, oerwrite number against *Run Time* by the number for the run time, e.g. 10. Press ***Close*** key, question: Save it? Yes

Opening the data acquisition window

Press Analysis-Chromatogram button (or ***Window, New, Chromatogram menu*** points),
in the **Selection of Data Channel and Serial Port** box
press circle after *Trace 1*, press OK

Loading a Method

Press met button (or menu points ***Method, Load***),
click in the **Load Method File** box in the list of files: TEST.MET, press ***Open***

The HPLC pump is started to pump the required solvent, wait until the pressure has stabilised and the column is equilibrated, autozero the detector. Then the sample is loaded into the injector loop. Simultaneously to turning the injector to the inject position the chromatogram is started by doing the following:

Starting the chromatogram:

Press Green light button (or menu points ***Operation, Start*** or F1 function key)

The chromatogram TEST0001 will be recorded.
Wait until the run time has elapsed,
A print-out of the chromatogram will be printed.

Recording the next chromatogram:

Inject the sample and simultaneously press the green light button (or menu points ***Operation, Start*** or F1),

the chromatogram TEST0002 is recorded
and the print-out be printed after the run time has elapsed.

Recording the next chromatograms:

Inject the sample etc. (TEST0003 etc.)

Calibration and quantitative Evaluation

All steps are carried out, no window is closed and a standard solution had been injected.

Creating a Calculations file

Click menu point ***Window***, line Edit Files,
Press cal button (or menu point ***Chrom.Files, Calculation***), in the **Open Calculation File** box overwrite UNTITLED after *filename* with TEST, click ***Open***,

Select *External Standard*, enter the concentration unit of the standard solution against *Calculation Units*,
Click index card ***Peak Table***, click ***Graphic*** key. In the **Select Report Files** box double click the name of the standard chromatogram in the list of files, press OK.
Mark peaks by dragging the mouse with pressed left key, press OK
Click the line of the peak table, enter the standard amount in the box under *Amt.in Std.*, enter the name of the solute under *Peak*,
Press ***Insert*** key, repeat for all peaks,
Press ***Close*** key, Save it? Yes.

One point calibration

Press balance button (or menu point ***Window, New, Calculations***),

Press Red point button (or menu point ***Calibration, One-Point-Calibration***),
in the **Select Calculation File and Report File** box choose in the list under *Calculation File* TEST.CAL and in the list adjacent the name of the standard chromatogram Test000x.RPT, press ***Open***,
in the **Select Level** 1 box press OK,

Press the printer button for the print-out of the results list (or menu point ***File, Print***), in the **Report Template File** box choose e.g. SHORT.RPF in the list of files, press ***Open***

Calculating the unknown amount

Press ±Vx button (or menu point ***Calculation***),
In the **Select Calculation File and Report File** Box click in the list under *Calculation File* TEST.CAL, in the liste to the right click the chromatogram name of the sample Test000y.RPT, press ***Open***, in the **Update Input Values** box press OK,

for the print-out of the results list press the printer button (or menu point ***File, Print***), in the **Report Template File** box click e.g. SHORT.RPF in the list of files, press ***Open***

5.2 Evaluating a Chromatogram

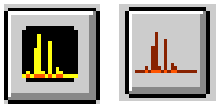
5.2.1 Automatic Reintegration

A stored chromatogram can be evaluated by automatic reintegration at a later stage. It is necessary to pay attention to the following when selecting the data handling file to be used:

1. Using ***Reintegration*** chromatograms are re-integrated with a data-handling file. The result of the integration is stored in a temporary file with a name where the first character of the original file name is substituted by a ~ sign. The result of the integration can be saved in the original report file – a warning appears that the file is overwritten – or in a new file. On leaving ChromStar the temporary files are deleted. Only one temporary file (.SLI + .RPT) is created for all subsequent reintegrations when an appropriate entry in the CHRST32.INI file is made. The last integration performed can be repeated with ***Reconstruction*** or in **Reprocess, Calculations** with ***Batch*** and Mode=Reconstruction.

2. The noise value should be suitable for evaluating the chromatogram. If nothing is known about it, a value between 100 and 500 is recommended. The integration parameters can be changed during the reintegration via the menu point ***Param***.,

The noise value in the Preset-File obtained by Automatic Noise determination and also the default value in the Data-Handling-File could lead to an unsuitable value being entered. Because of this the entries should be tested. The noise value can be modified at a later stage by means of Param.



To perform an automatic reintegration click the menu point ***Integration*** followed by ***Reintegration*** in the **Reprocess** window. Select a chromatogram and a data handling file from the directories. In a preview the chromatogram is displayed in the small box to the right, showing the on top *Sample Identifier* if an entry in the *Sample Table* of the method file had been made After clicking OK the calculation is carried out and the reintegrated chromatogram appears on the screen. The name of the chromatogram starts with the tilde sign.

Several options are available to present the result.

With ***Annotate*** peak numbers, retention times, individual descriptions or peak names from the relevant calculation file can be written into the chromatogram.

If the chromatogram is to receive the peak names defined in a peak table of a calculation file, the name of this calculation file must be entered in the data handling file against Calculation File. The method of calculation specified in the calculation file will be carried out.

A manual annotation can be saved in the report fila via ***Files, Save***.

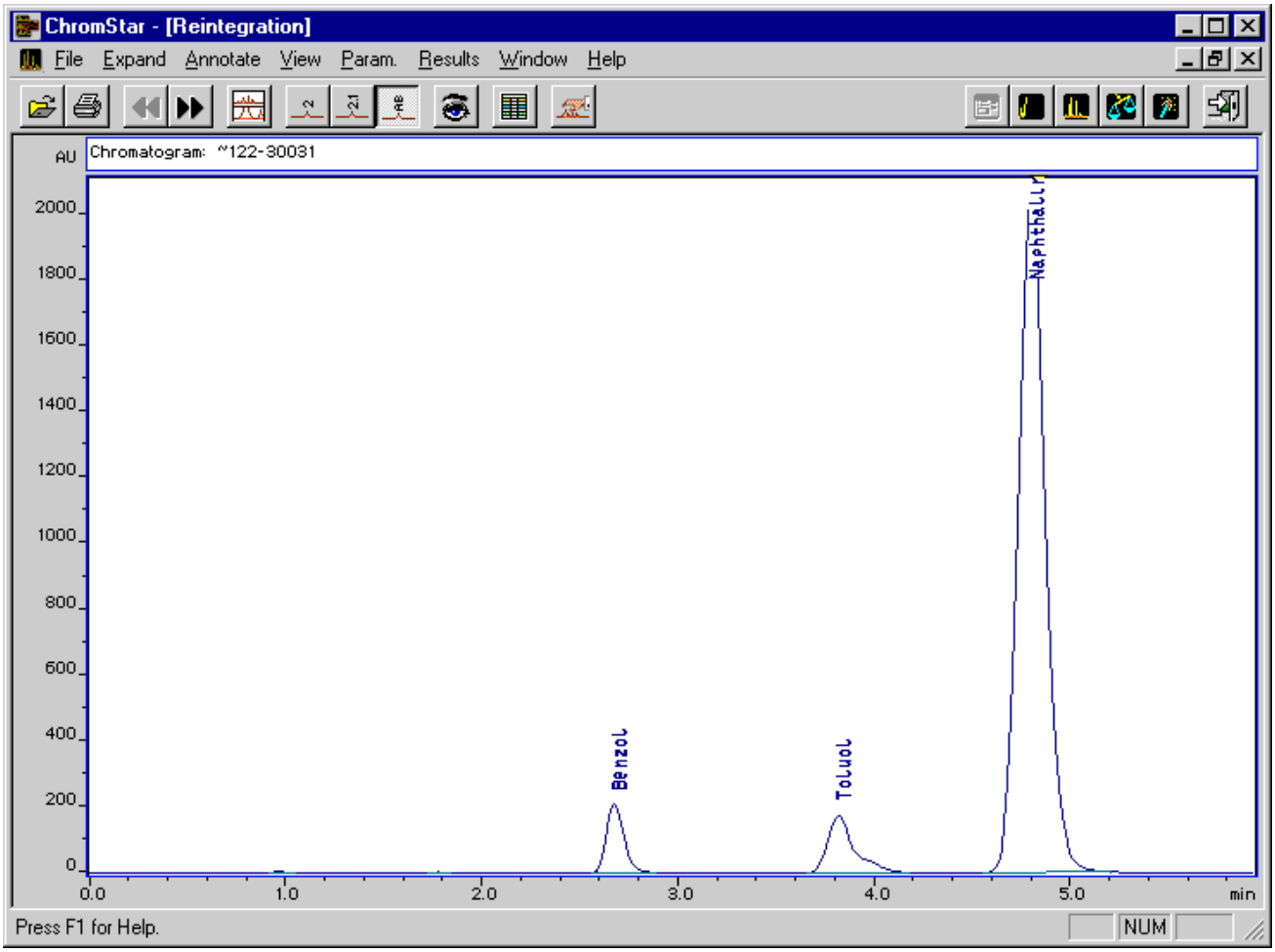
With ***Files*** and ***Print*** and selecting a report print template (.RPF) the chromatogram can be printed. A preview of the print-out appears in the box to the right.

In order to judge the reintegration an enlarged section of the chromatogram can be displayed by means of ***View*** and ***Split*** or ***Expand...***

If the result is not satisfactory the time programmed integration parameters can be redefined via the menu point ***Param.*** The calculation with the changed parameters is executed after clicking OK.

If suitable parameters are obtained in this way, these can be entered in the Data-Handling-File by clicking *Param*. and *Save*.

If sections of the chromatogram have still not been satisfactorily integrated then the menu point ***Files, Manual-Integration*** can be used to perform a manual integration.



5.2.2 A Description of some Integration Parameters

5.2.2.1 The Influence of the Noise Value on the Integration

The noise value (1 to 9999 in µV/sec) determines the minimal increase of the detector signal which is still judged as peak start. Thus the first derivative of the chromatogram is examined, peak start is determined if the increase is greater than the noise value for 6 consecutive data points. The peak start is then plotted to the first of these points. The peak end is determined as soon as the increase of 6 consecutive data points is lower than the noise value. In this case the peak end is plotted to the last of these points.

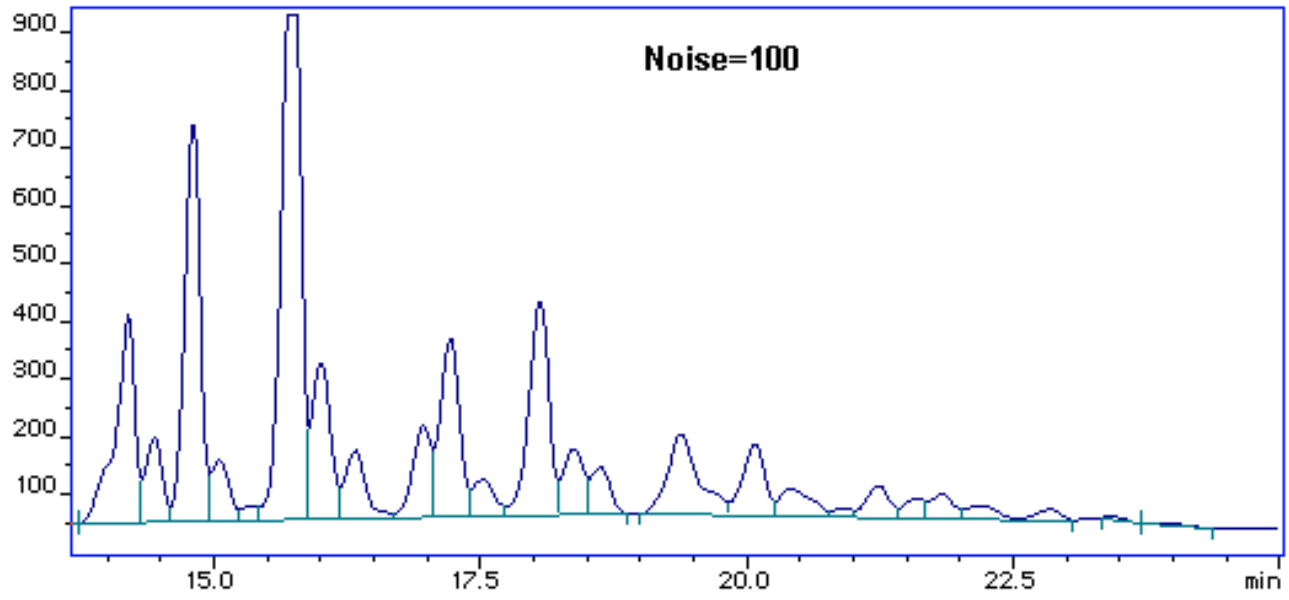
Peak recognition is also influenced by the slice width because the examined time interval of the detector signal function is dependent on the slice width. Peak recognition will obviously not function satisfactorily if the slice-width is defined so small that peaks are recognised in the noise of the detector.

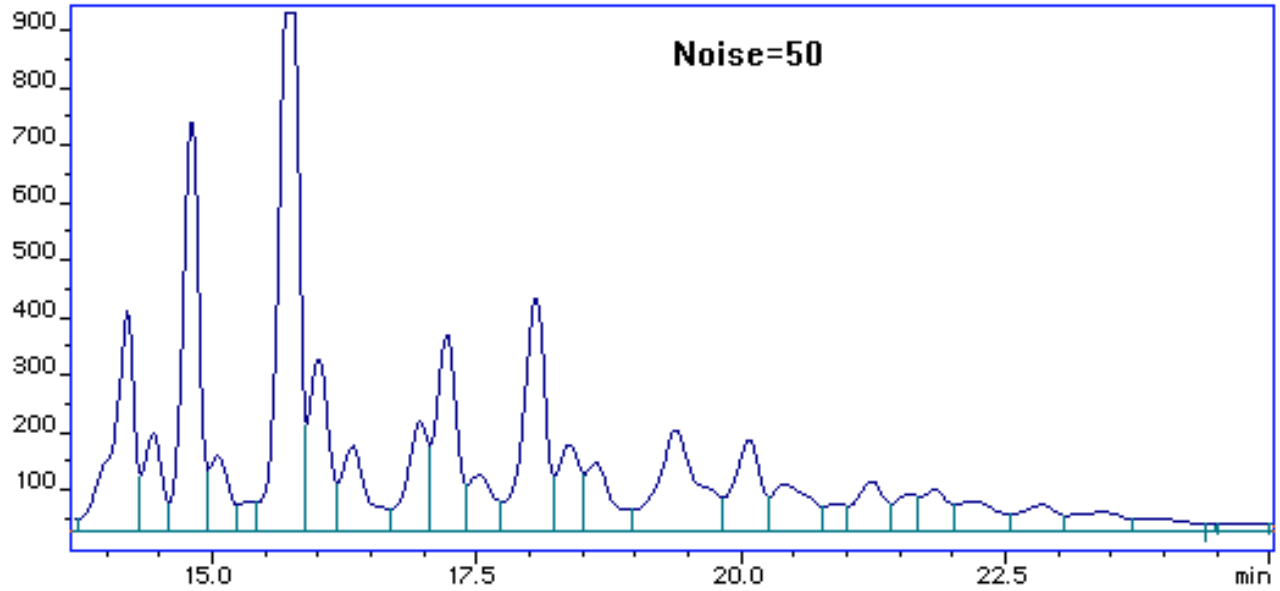
The number of points, the slope vaue of which is to be averaged, can be increased by the Peak Parameter (max. 15). Then even in a great signal/noise ratio peaks can be recognised.

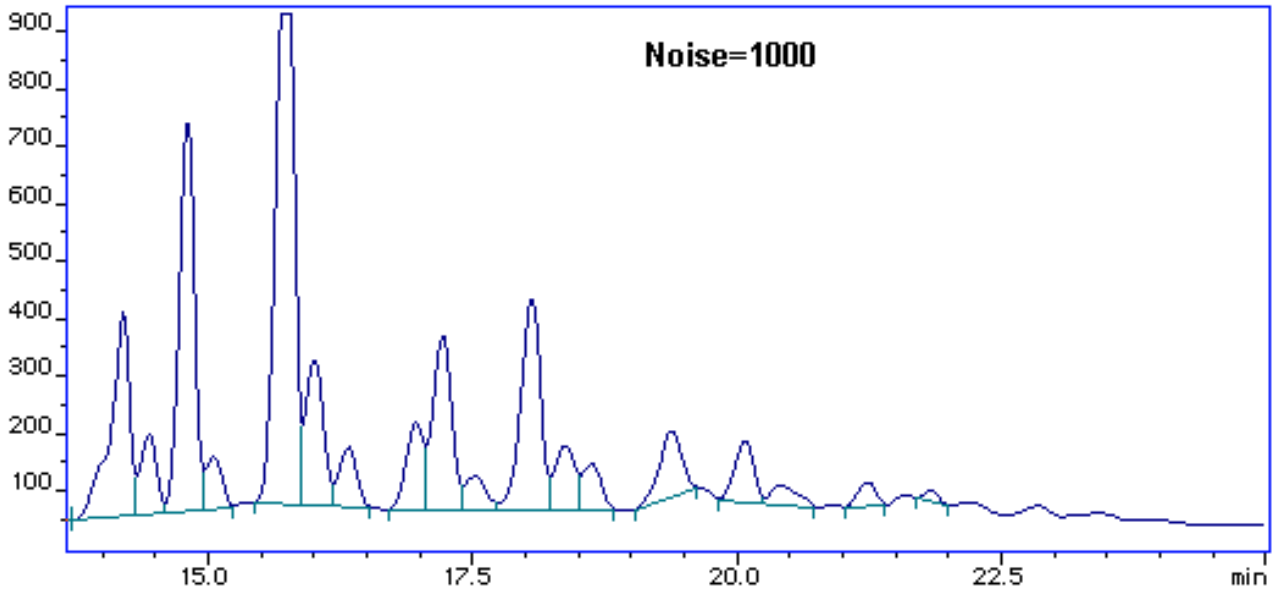
The application **Analysis** offers the option to determine the noise value automatically with ***Control*** and ***Automatic Noise*** in the draw baseline window. This avoids peaks to be detected in the detector baseline. This value is automatically transferred to the preset table and is used for all integrations if no other noise value has been defined in the data handling file for time = 0.

During automatic noise determination the slice width defined in the preset table is used. If possible this should be set at the same value as selected for recording the chromatogram.

If the determined noise value proves to be suitable for the integration it should be stored in the time table of the data handling file at time = 0. With that it is available for further evaluations and will not be overwritten by new Automatic Noise determinations.







In the example on the left integration has taken place with noise values of 50, 100 and 1000. Obviously, the value 1000 is too high to allow satisfactory peak recognition. The value 50 prevents the recognition of a correct peak end, because of this the baseline end is fixed to far backward and the peaks areas are too large.

5.2.2.2 The Use of the Skim Ratio

The integration parameter *Skim Ratio* is used to perform a differentiated integration of two overlapping peaks with a common valley.

First the ratios a/b and c/b are determined in which a and c are the heights and b is the common valley height of two adjacent peaks. This relationship is analysed for

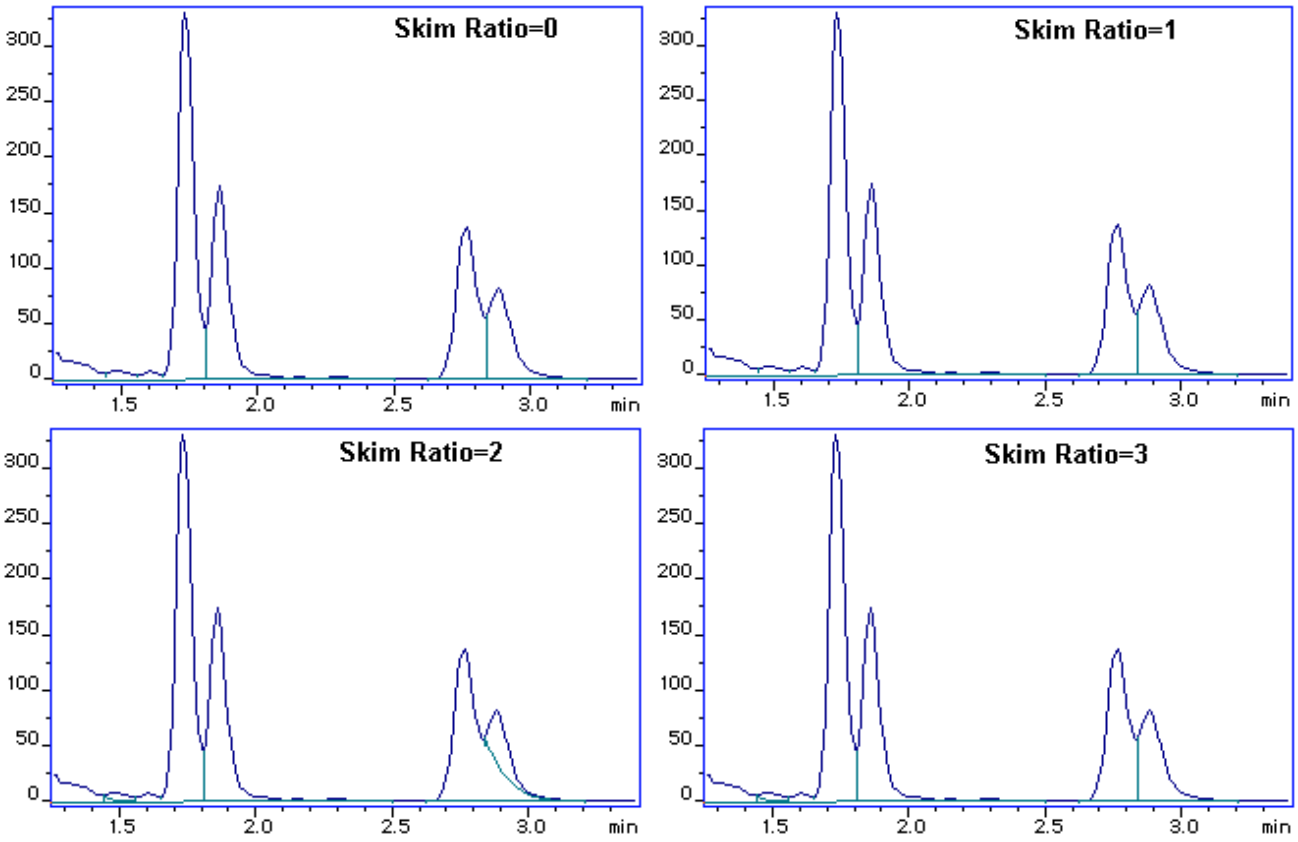
a/b >> Skim Ratio >> c/b

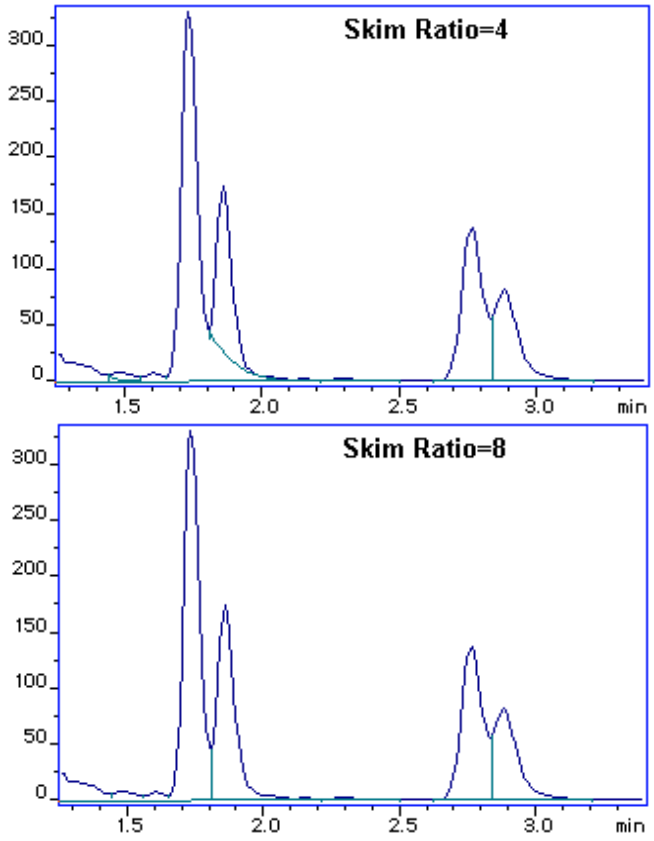
If the skim ratio value lies between these two values an exponential skimming is carried out i.e. the lower of the two peaks is treated as being on the flank of the higher. The skim function plots the presumed shape of the higher peak. The area calculation of the peak on the flank is performed up to this line.

If the Skim Ratio value is outside of both values the peaks are separated by a perpendicular drop line.

A Skim Ratio value of 0 also results in peak separation by perpendicular drop lines.

The following figures show the effect of the value of the Skim Ratio parameter on the integration of two peak groups consisting of overlapping peaks with different height ratios. The value is changed from 0 to 8.



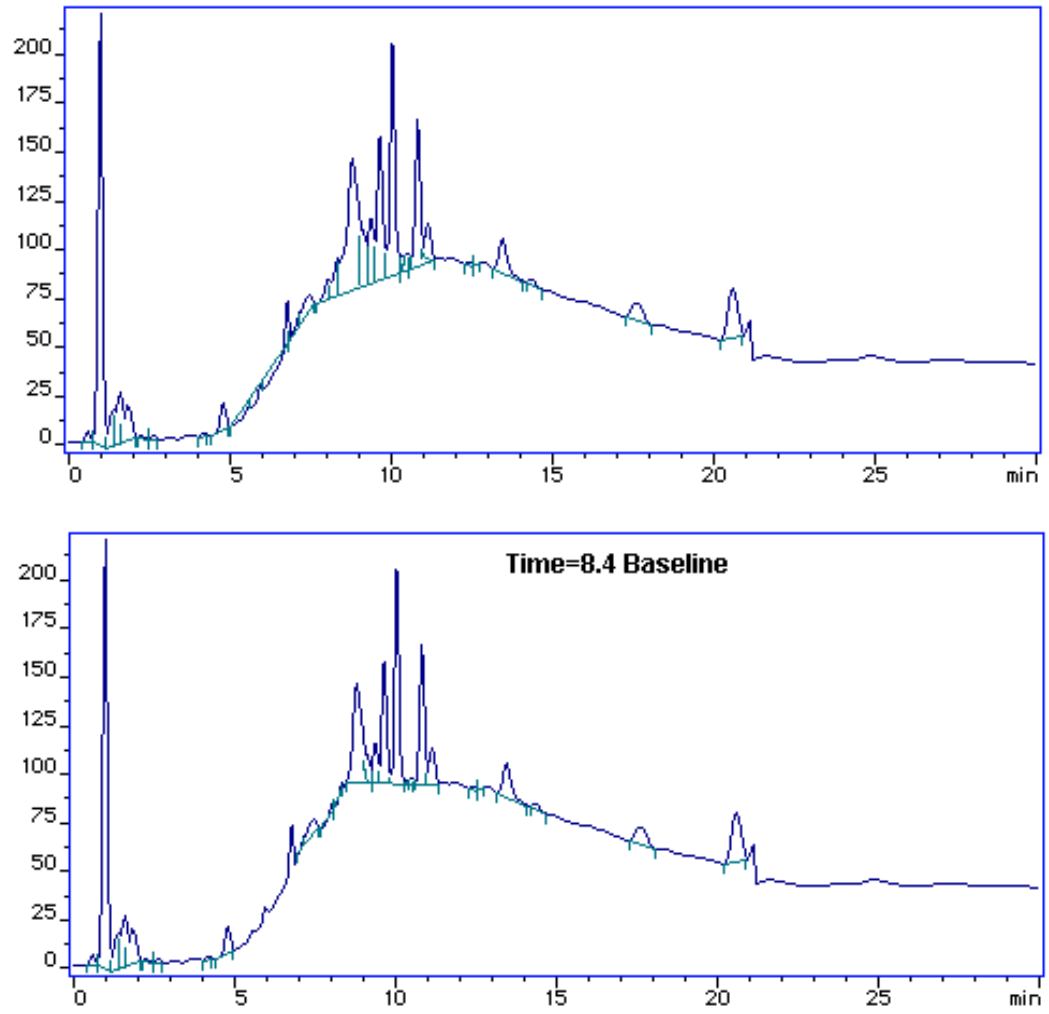


Skim Ratio-values from 5 to 7 show the same result as for 4. A subpeak situated on the left (rising) flank of a main peak is always separated by a perpendicular drop line unless the subpeak is only a shoulder. Only in manual integration it is possible to calculate a small peak separated by a valley on the left flank of a larger peak by drawing a skim function.

5.2.2.3 The Baseline Parameter

With the parameter Baseline a new start point of the baseline can be defined. This function is always necessary when the baseline of the detector signal has changed considerably during a short period of time e.g. after a change of wavelength, a change of eluent or in a gradient run as in the example shown.

In the example the integration was prevented using Integrate Inhibit during the period of the increase of the detector signal.

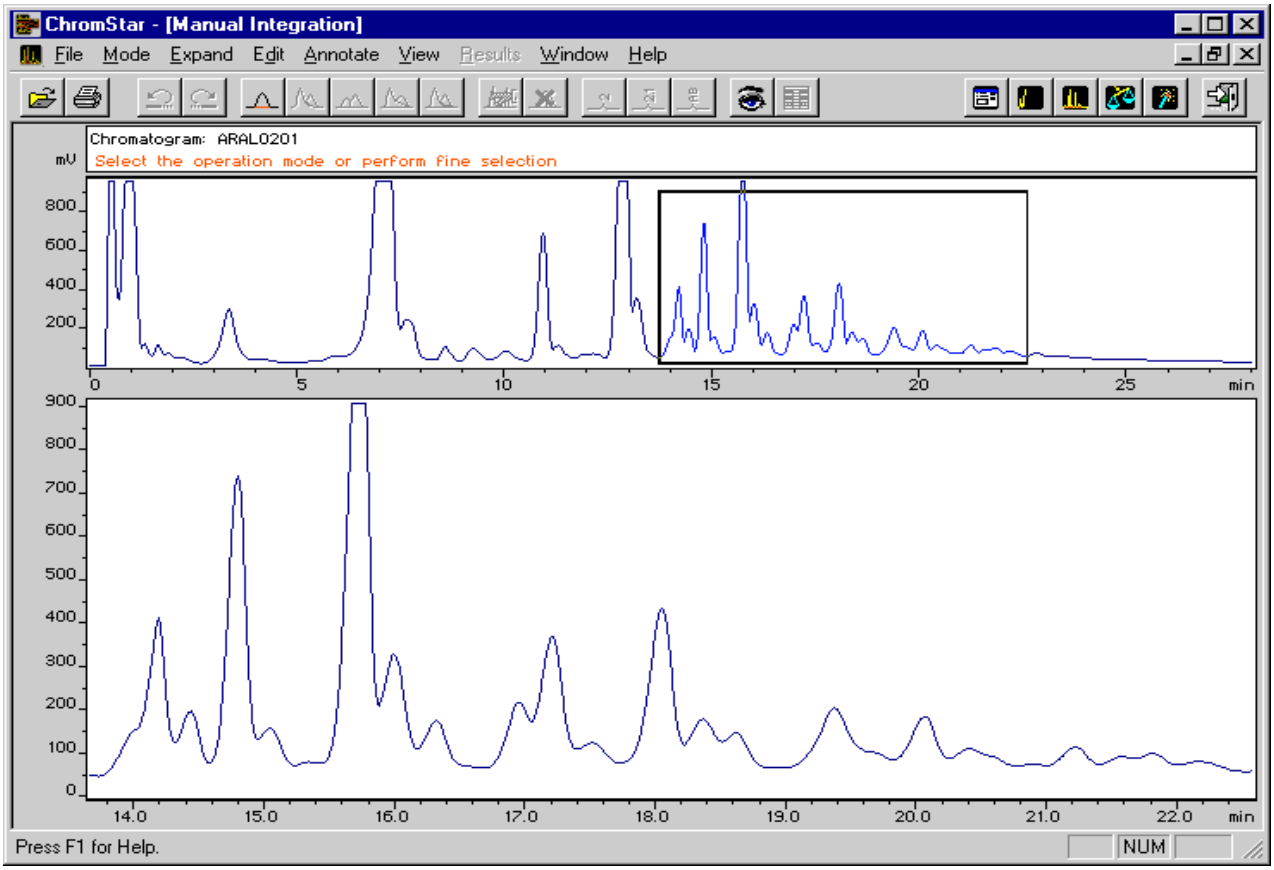


5.2.3 Manual Integration

If a data handling file with unsuitable parameters for evaluation was used when recording the chromatogram, the peak data can be obtained later by manual integration of the chromatogram. For that purpose click the menu point ***Integration*** and its submenu point ***Manual Integration*** in the **Reprocess, Integration** window.

Select the desired chromatogram by clicking it in the list of stored chromatograms.

Select a section of this by constructing a rectangle in the upper part containing the peaks to be evaluated.



Select the submenu point ***Tangential Baseline*** in ***Mode*** and draw the baseline in the enlarged chromatogram section by clicking the left button at the desired baseline start, drawing it, clicking the left button again and confirming with the right button. The baseline is drawn as a straight line across the selected range.

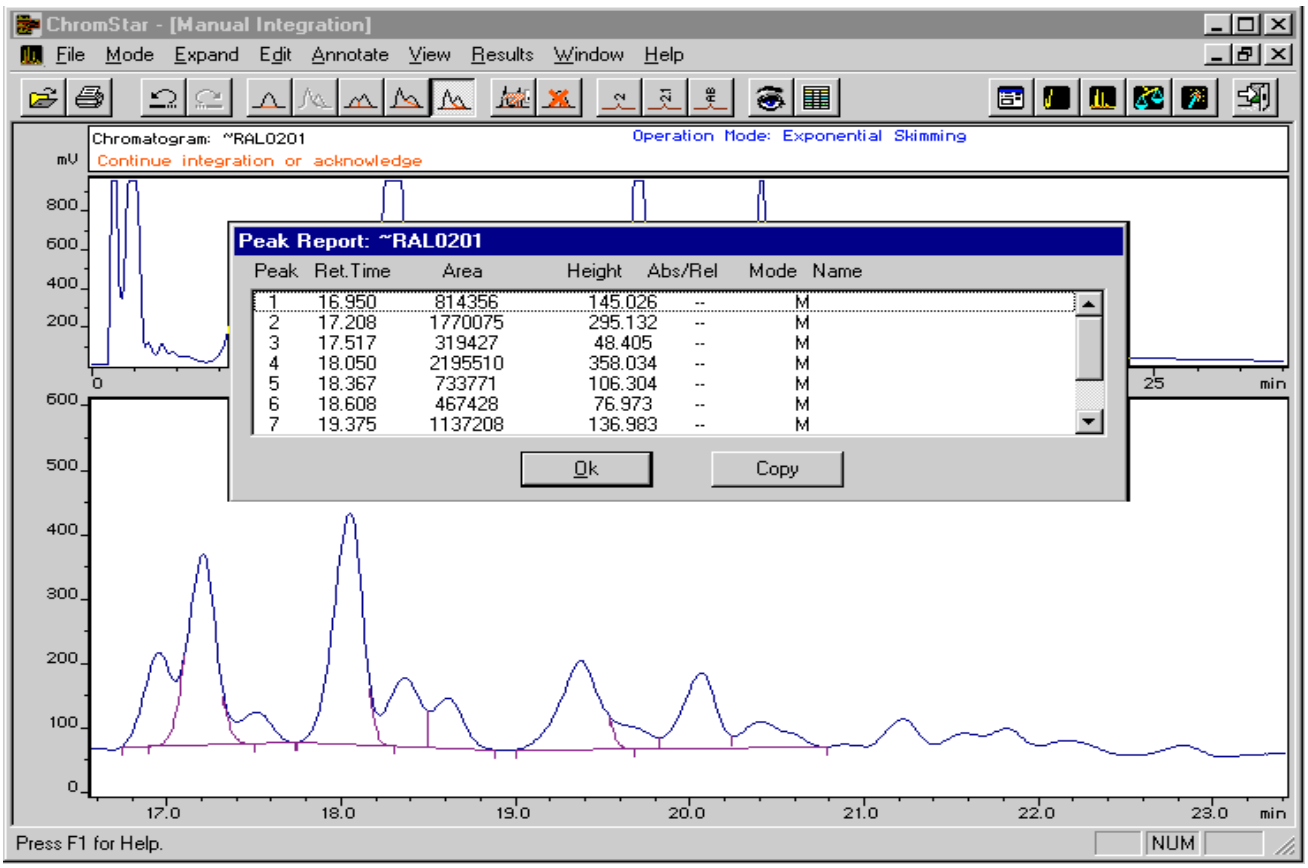
This can be done more times in succession. Further peak separations can now be made in the enlargement by constructing perpendicular drop lines with ***Valley*** or by drawing of overlapping peaks with ***Exponential Skim***. For a simple peak it is only necessary to draw the baseline.

In order to have a precise check on the retention times the ***View*** function can be employed when defining peaks, with its aid the retention time and the mV signal at the cursor position are displayed.

The evaluation of the peaks is started by clicking the right mouse button in the white area of the upper chromatogram. This completes the manual integration in this section. The peak separations made are drawn in the upper part and the lower part is cleared. After constructing a new rectangle the following operations can be performed.

With ***Annotate*** peaks can be shown with retention times. In the lower section peaks can be annotated by individual entries.

The results list appears on the screen by clicking ***Results***.



The results can be presented or viewed in various ways.

With ***File*** and ***Print*** the chromatogram and the results list can be printed after choosing a report print template.

Save stores the result of the manual integration as a Report-File (.RPT). A warning appears that the original report file will be overwritten. The chromatogram and the integration saved in the Report-File can be displayed in ***Reprocess, Reconstruction***. The Report-File can be used for quantitative evaluations.

5.2.4 Grouping and Summation of Peaks

It is possible to evaluate peaks in a chromatogram in groups i.e. the areas of several peaks are summed, this total is used to calculate area percentages or amounts..

Peak grouping is used to add adjacent peaks. The identification of these peaks is done in the peak table of a calculation file by creating a time window around a central peak and marking against Code with G. The areas of all peaks occurring in this time window are summed. A chromatogram can contain several time windows identified with G.

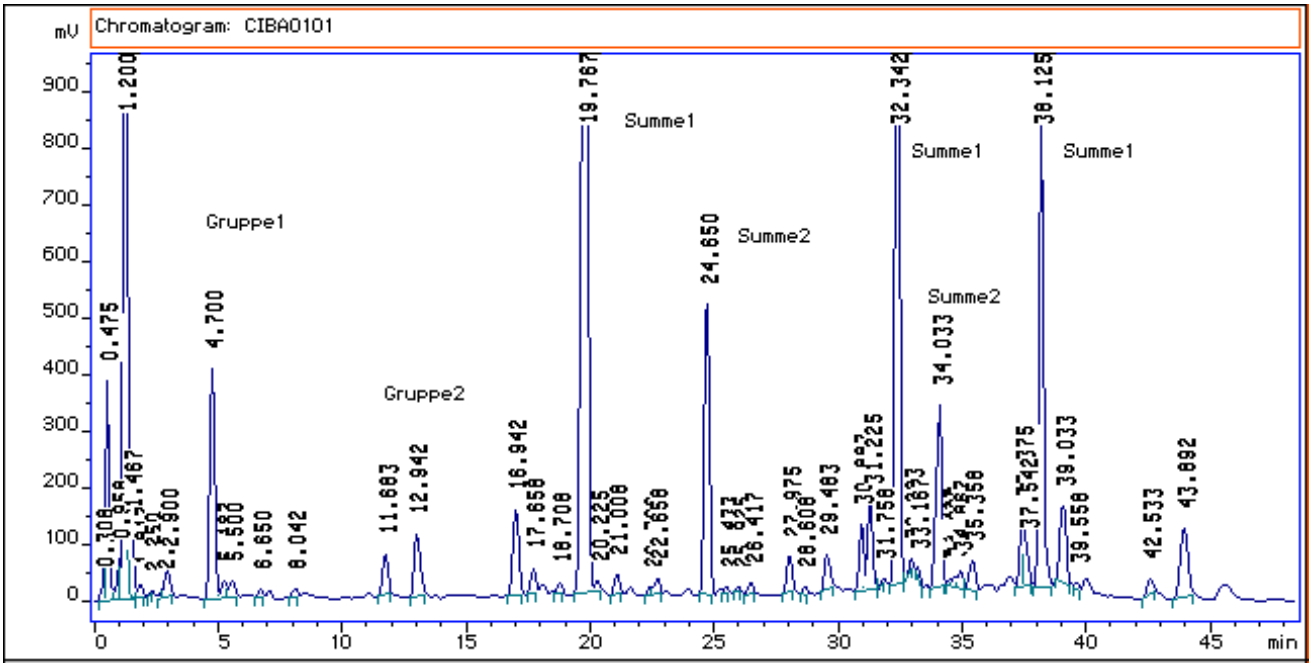
Peak summing allows you to select non-adjacent peaks in a chromatogram for joint evaluation. These peaks are identified in the peak table of the calculation file against Code with S1.

A further peak area sum of other peaks can be obtained after identification of these peaks as S2, etc

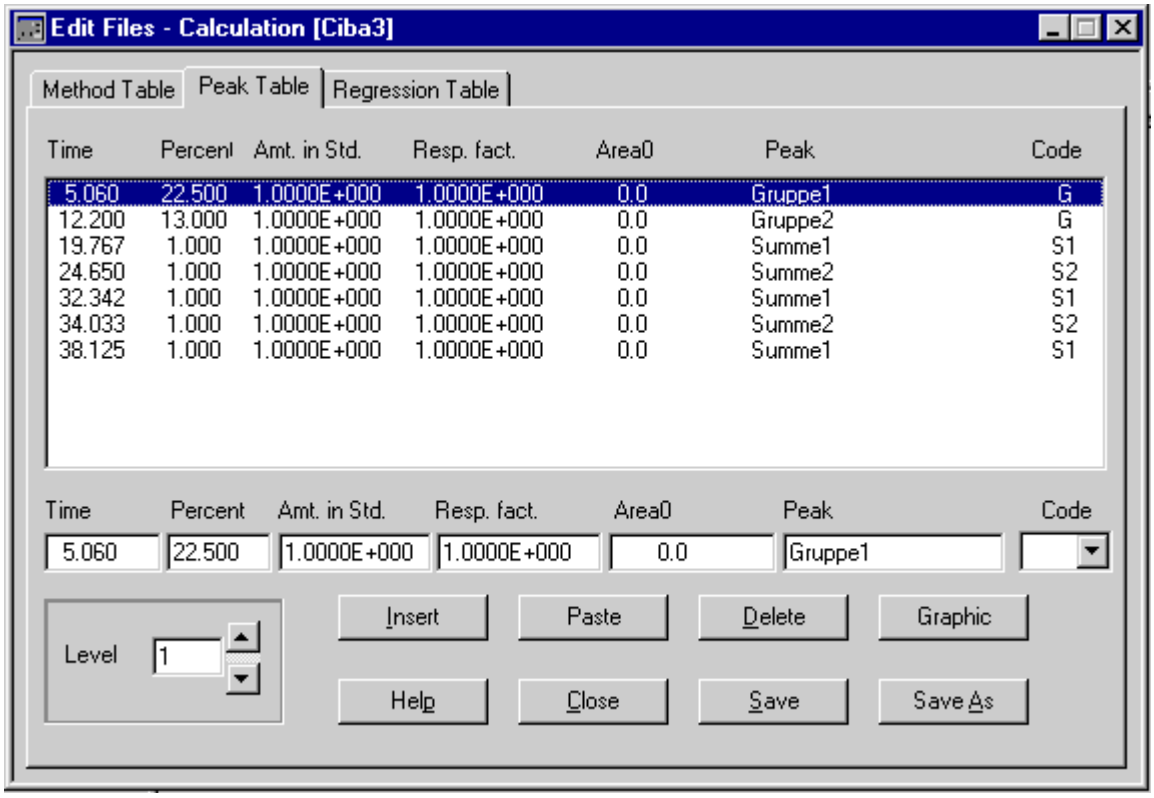
In the following example (chromatogram CIBA0101, results list without summing or grouping **17**) the peaks from 4.0 to 6.12 min. are evaluated as one group and the peaks from 10.71 to 13.76 min. are evaluated as a further group.

The peaks at 19.77, 32.34 and 38.13 min. are evaluated as Sum 1 and the peaks at 24.65 and 34.03 are evaluated as Sum 2.

The annotation in the chromatogram was done manually.



For this purpose a calculation file is created with the following peak table.

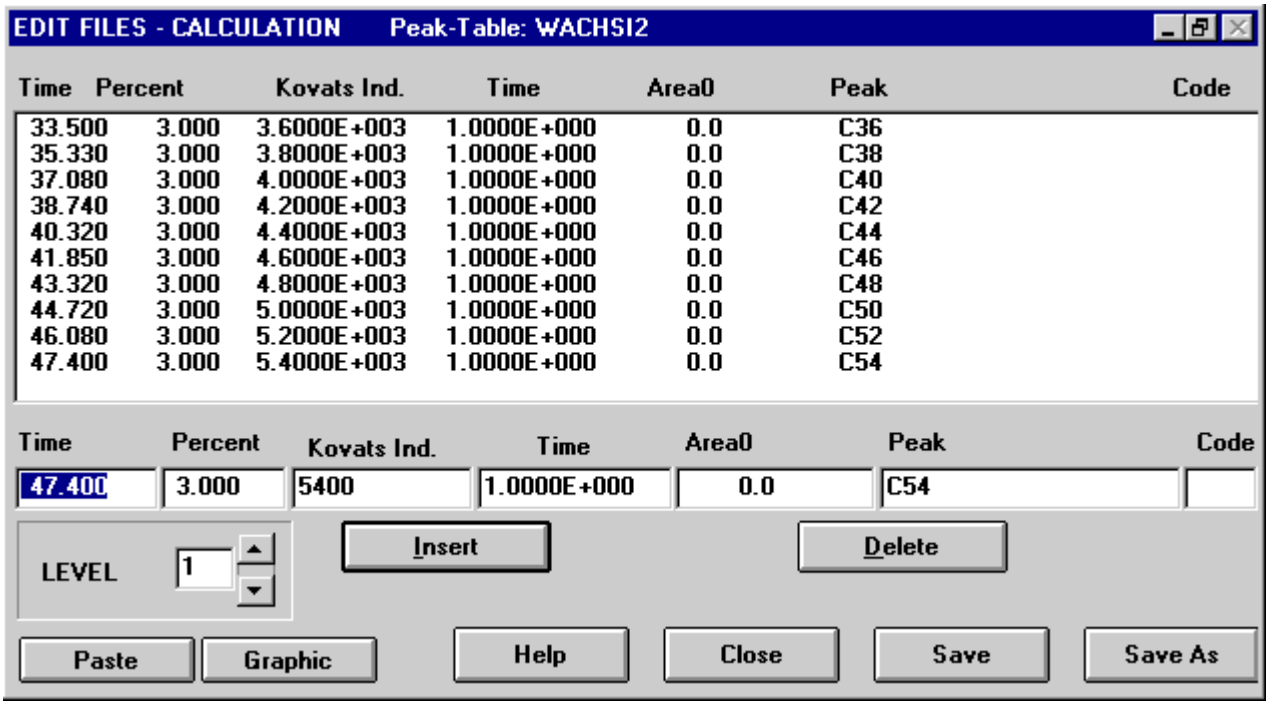


The name of this calculation file is entered into the data handling file CIBA.INT. The evaluation (**18**) contains in case of peak grouping the average retention time of the peaks in the Group, in case of peak summing the retention time of the first peak of the corresponding Sum is presented. In case of summing the individual peaks will not be listed in the report.

5.2.5 Calculation of Kovats-Indices

The Kovats-Index represents the relation between carbon content and retention time of a substance in a gaschromatographic system.

The determination can be performed with an internal standard as well as an external standard. The standard to be used is a series of homologous hydrocarbons. For this calculation a Calculation-File is created which has marked as method on the first page Kovats intern or Kovats extern. Time windows are defined in the peak table. The Kovats-index of the standards are entered against Kovats Ind. and the retention times of the standards are entered against the following Time-field. The name of the Calculation-File is entered in the Data-Handling-File to be used..



In the Internal-Standard-Method the standard is added to the sample and a chromatogram is recorded. The retention time of the standard must be obtained before.

In the External-Standard-Method a chromatogram of the standard is recorded first, the retention times obtained in this way are entered into the Calculation-File. After this the sample is chromatographed.

The dead time is determined by a first order linear regression through the standard points.

The Kovats-Indices of the peaks found in the time windows are calculated according to the following formula:

LINp=(lg(RTp)-lg(RTss)) * (LINps-LINss) / (lg(RTps)-lg(RTss)) + LINss

For a peak before the first standard applies:

LINp=lg(RTp)*LINss/lg(RTss)

For a peak after the last standard applies:

LINp=lg(RTp)*LINps/lg(RTps)

in which:

LINp = Index of the peaks

LINss = Index of the next standard peak

LINps = Index of the previous standard peak

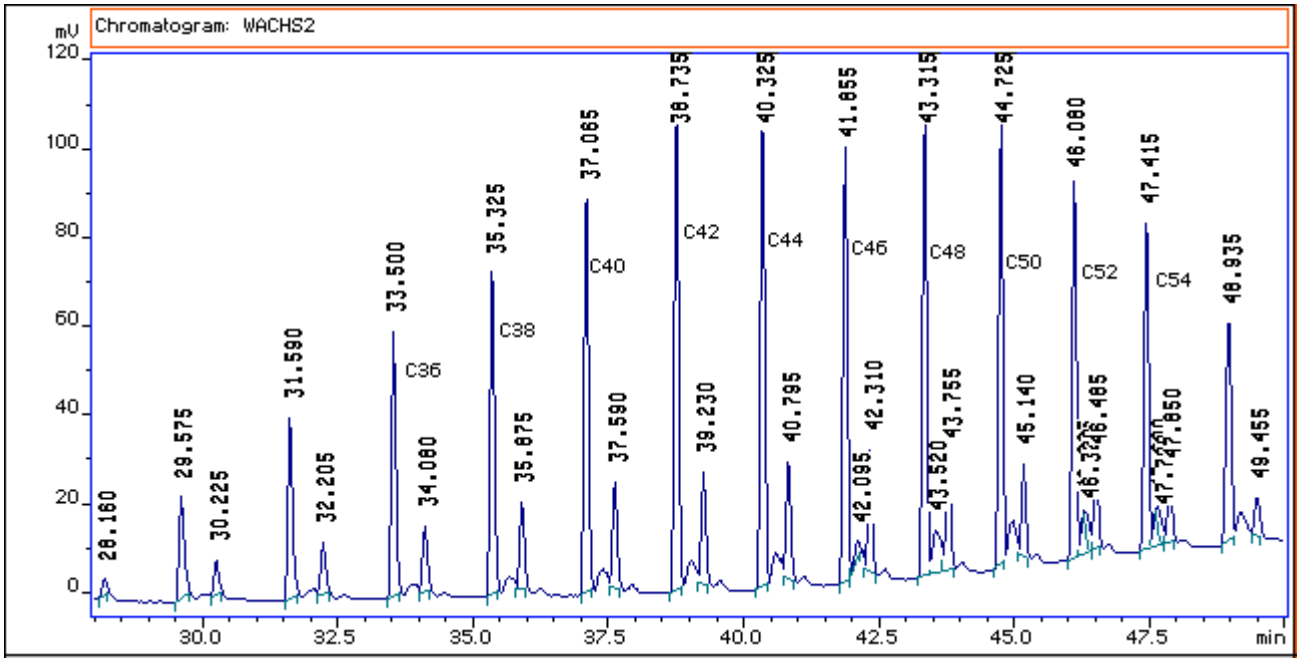
RTp = reduced retention time of the peaks

RTss = reduced retention time of the next standard peak

RTps = reduced retention time of the previous standard peak

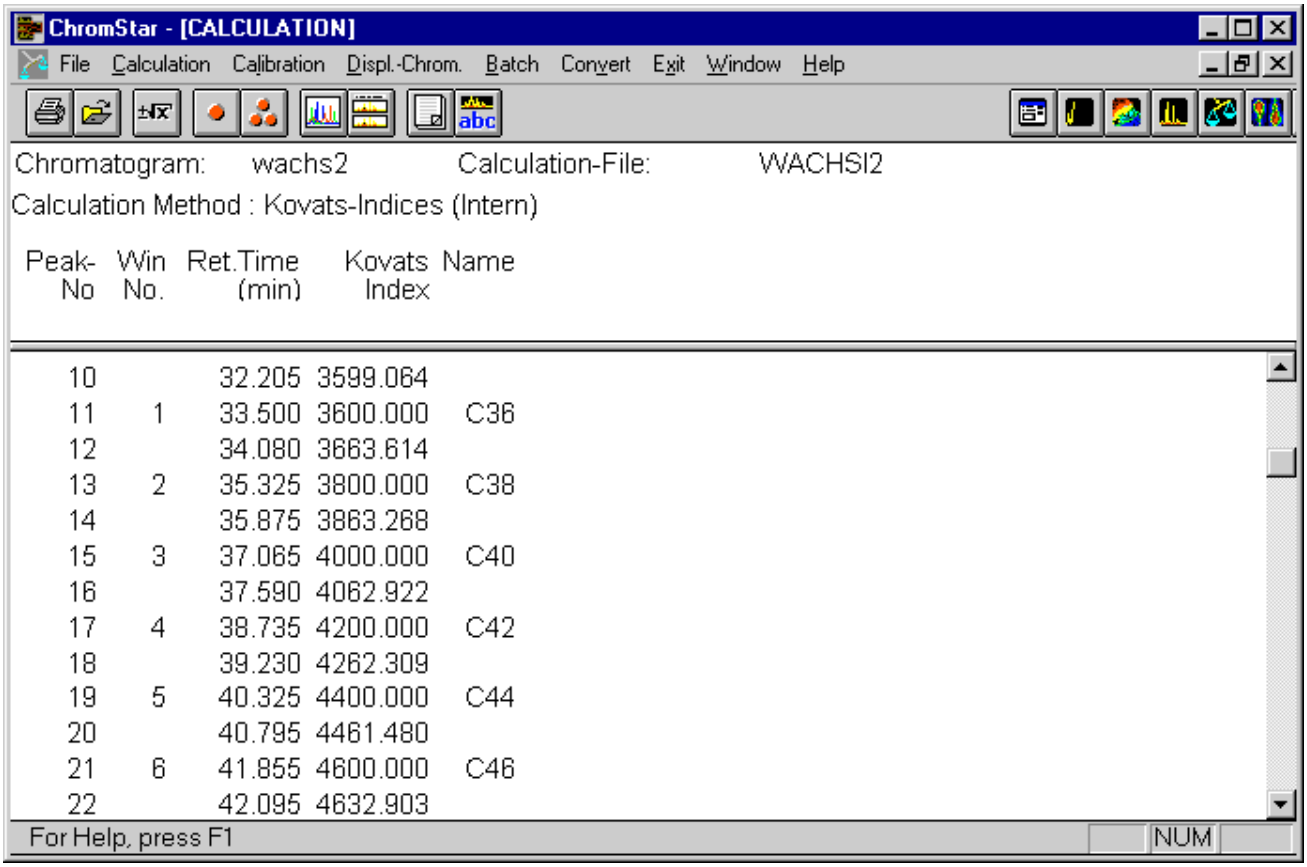
The calculation is done either in **Reprocess**, ***Reintegration*** using a data handling file with the name of the calculation file mentioned above or in **Reprocess**, ***Calculations***.

The following chromatogram was evaluated with the Internal Standard method.



The standard peaks were annotated manually.

The results list after evaluation in **Reprocess, Calculations** shows under *Win* the number of the time window, the retention time of the standard peak, the Kovats Index entered and under *Name* the description. For all other peaks, for which the peak area is greater than the area defined in the Calculation file against *Minimum Area*, the Kovats Index will be calculated.



5.2.6 Column Coefficients

Various column coefficients are used to describe the performance of a column, such as theoretical plate number, plate height, capacity factors and coefficients of selectivity. These coefficients should be tested from time to time by means of a chromatogram of a test mixture.

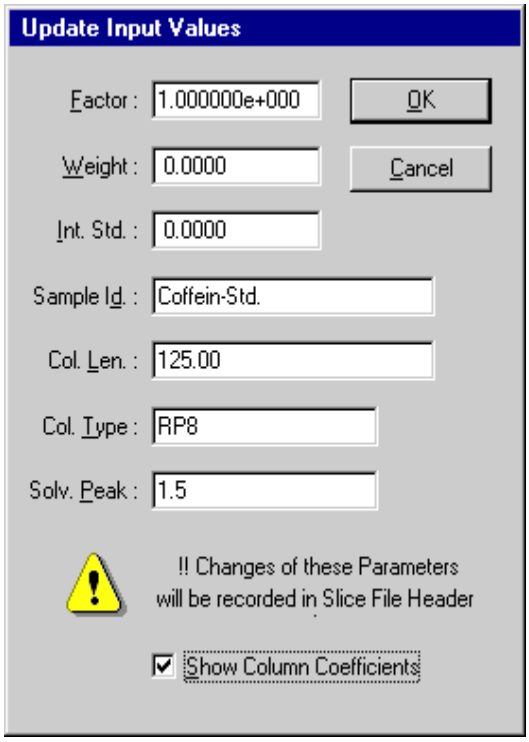
The calculation needs the following parameters:

- Column length L in mm
- Retention time of a non-retended substance T_0 in min (e.g. solvent peak) (RT-Solv)

The following coefficients are calculated:

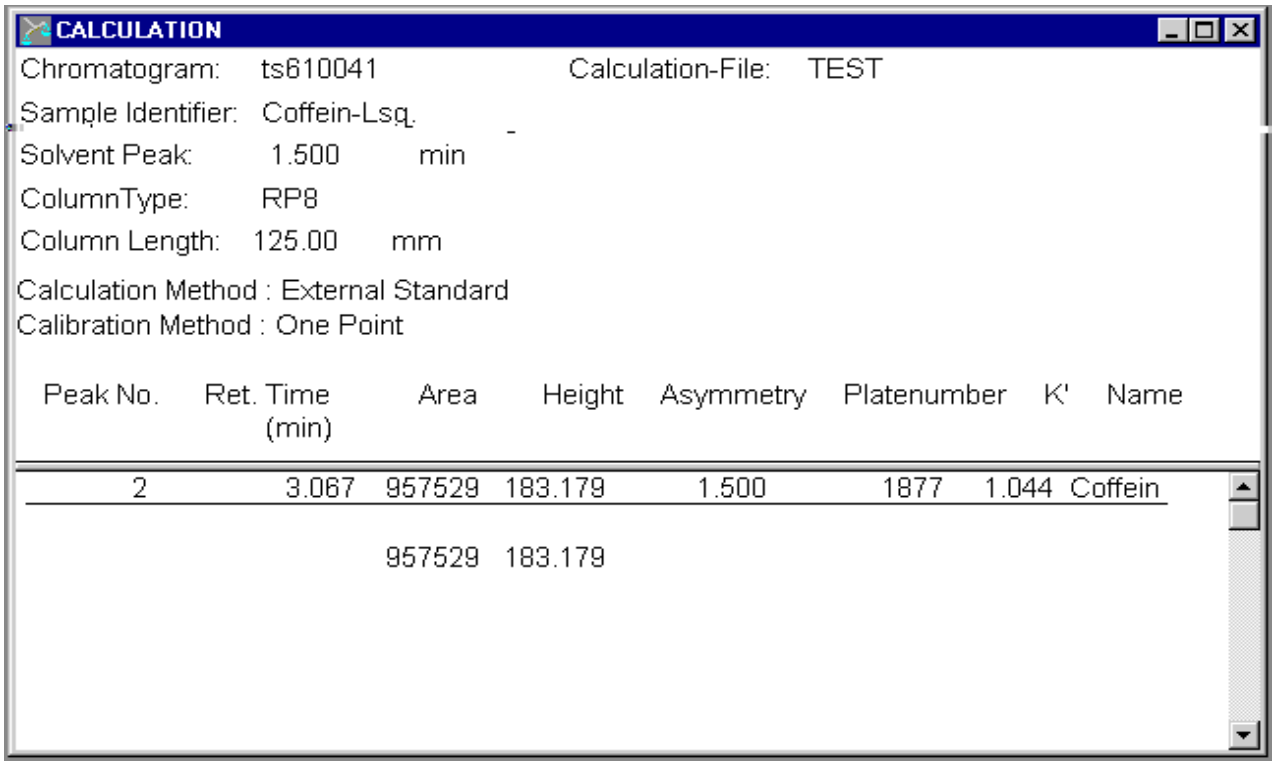
- Linear velocity u
- Theoretical plate number N (Th Plate No)
- Plates per meter
- Plate height (HETP)
- Capacity factor k'
- Asymmetry factor A
- Selectivity a
- Half height peak width W0.5
- Resolution R

The calculation of the column coefficients is carried out immediately after recording the chromatogram, if the CHRST32.INI file contains the entry Calculation=Standard in the chapter [Colcoeff]. When the entry is Calculation=Disabled or when there is no entry, no column coefficients are calculated. The preset values for the parameters used for calculation are column length = 125mm and solvent peak = 1 min. These values are entered in the method file in the documentation page and can be changed before recording the chromatogram as required.



The calculation can be carried out at a later time in ***Reprocess, Calculation***.

After choosing the chromatogram and a calculation file the values for column length and solvent peak are entered in the box **Update Input Values.** After clicking *Show Column Coefficients* the results list showing the column coefficients appears in the **Calculation** window. For the print-out an appropriate print report template must be used. For details cp. Chapter 4 in the Report Editor manual, objects *Column Details*, *Linear Velocity*, *Solvent Peak* and *Table*, *Available Items*.


CALCULATION

Chromatogram: ts610041 Calculation-File: TEST

Sample Identifier: Coffein-Lsq.

Solvent Peak: 1.500 min

ColumnType: RP8

Column Length: 125.00 mm

Calculation Method : External Standard

Calibration Method : One Point

Peak No.	Ret. Time (min)	Area	Height	Asymmetry	Platenumber	K'	Name
2	3.067	957529	183.179	1.500	1877	1.044	Coffein
		957529	183.179				

The column coefficients are calculated by using the formulae:

Linear velocity:	$u\ (mm/s) = L / (T_0 * 60)$
Plate number:	$N = 5.545 * (T/W)^2$
Plates per meter:	$N_m = N * 1000 / L$
Plate height HETP (or H):	$H\ (\mu m) = L * 10^3 / N$
Capacity:	$K' = (T - T_0) / T_0$
Asymmetry:	$A = w_b / w_f$
Selectivity:	$a_{(i+1/1)} = K'_{(i+1)} / K'_{(i)}$
Resolution:	$R_{i+1,i} = (T_{i+1} - T_i) * 2 / (W_i + W_{i+1})$

wherein is:

L = column length (in mm)

T_0 = Retention time of solvent peak (in min)

T = Retention time (in min)

W = Half height peak width (in min)

w_f = Peak width in 10% of peak height from the ascending peak flank to the perpendicular

w_b = Peak width in 10% of peak height from the perpendicular to the descending peak flank

w_f and w_b are calculated in 5% of peak height, if CHRST32.INI shows the entry Calculation=FDAComp in the section [Colcoeff].

T_{i+1} , T_i : Retention times of two adjacent peaks

F= Factor, as specified in CHRST32.INI in [Colcoeff] against Factor, preset is Factor=2.

5.3 Quantitative Calculation

5.3.1 Calibration methods

The various calibration methods allow you to determine the response factor for calculating an unknown amount from a peak area or height or to define a calibration function for the relation between amounts and peak areas.

5.3.1.1 One-Point Calibration

In the one-point calibration a chromatogram is recorded of a standard solution with known quantities of all compounds which have to be determined later. Retention times, peak areas and heights of the compounds are obtained by integration.

The calculation file to be used for the calculation contains entries on the first page defining whether areas or heights are to be used and in which unit the concentration has to be stated.

The second page contains entries about the retention times with the corresponding time windows, the amount of the compound in the standard solution and the peak names.

The calibration is performed in the **Reprocess - Calculations** window by clicking ***Calibration*** and ***One-Point-Calibration*** and entry of the Report-File and Calculation-File.

The result appears in a table on the screen. The obtained response factors appear against Res.Fct. and are automatically transferred to the calculation file. Also a compound which is later to be used as an internal standard must have its response factor established as described. This compound has to be identified as an internal standard.

When carrying out a one-point calibration in the current run the name of the Calculation-File must be entered in the Data-Handling- File. The injection is identified as a calibration run by entering "S" against Type in the Method-File.

If standard solutes may not be mixed in one solution and one-point calibration are to be carried out with chromatograms of the single standard substances, in CHRST32.INI in the section [Calibration] there must be the entry Calculation=additive, so that the response factors of the substances not found in a standard solution is not set to 0. This is only valid for one-point calibrations.

5.3.1.2 Averaging a number of One-Point Calibrations

The response factors obtained from a series of injections of the same standard solution can be averaged in a current run as well as afterwards.

When calculating averages during a current run, mark Autosampler on the first page of the Method-File (also if no autosampler is used). It is always necessary in calibration runs to use a virtual autosampler. The injection of the calibration sample is identified in the autosampler table with S against Type, the number of injections to be performed is entered against Inj. The name of the Calculation-File containing the

Peak-Table is entered in the Data-Handling-File to be used.. The response factors established after each injection are stored until all injections of the calibration sample are carried out. After this the average values of the individual standard are calculated and entered into the Calculation-File.

The Calibration-Table (**11**) one receives contains a report of the average calculation.

To calculate the averages afterwards, first One-Point-Calibrations have to be performed with the Report-File in the **Reprocess – Calculations** window. Then click the point ***Calibration*** and ***Multi-Calibration***. Select the files obtained before from the list of Report-Files and click OK. The result of the average calculation with the standard deviation appears on the screen for the first peak of the Peak-Table. The averages of all other peaks are similarly calculated.

The results must be stored by means of ***File*** and ***Save Results***, herewith the calculation file used for the one-point calibration can be overwritten or a new file can be created.

Automatically entered in the new Calculation-File are the complete peak table of the calculation file used for the one-point calibration and the averages of the response factors in the regression table against K1.

First a warning appears if the original file is to be overwritten. The file is overwritten after clicking Yes.

5.3.1.3 Multi-Level-Calibration

The multi-level-calibration establishes a correlation between the concentration of a substance and the peak area and, from this, to compute an approximation function. Later with its aid an unknown amount can be calculated from a peak area. The multi-level-calibration can be performed in a current run as well as afterwards.

The execution in a current run is carried out either with a real or a virtual autosampler. Autosampler is marked on the first page of the Method-File.

The autosampler table contains in **one** line the series of vials with the calibration solution (the start number against Vial, the last number against To, a continuous series must be available).

The number of injections from each vial is entered against Inj., and S is entered against Type. The Data-Handling-File containing the name of the Calculation-File

to be used is entered against DH.

Method=External Standard is marked on the first page of the Calculation-File, the units of concentration are entered against Calculation Units and the number of different calibration solutions against Conc.Level (this corresponds with the number of vials). On the second page the different amounts of each peak in the calibration samples are entered. Therefore it is necessary to know the retention times of the substances to be determined.

The calibration samples are injected (also repeated, depending on the entry against Inj. in the autosampler table) one after the other after recalling the method in **Analysis**.

Calculation of the coefficients of the approximation functions follows automatically. These are transferred to the regression-table of the Calculation-File and used for the quantitative evaluation of the next sample (also if no autosampler is used) in the autosampler-table.

If the Multi-Level-Calibration is to be performed afterwards, first a number of chromatograms to be evaluated have to be recorded with calibration samples containing different amounts, also multiple injections of one calibration solution are permitted. These are integrated in order to obtain peak data.

Also a Calculation-File is created which contains on the first page the Method=External Standard, against Conc.Level the number of different calibration solutions and against Calculation units the unit of concentration. By using the Level-Entry in the peak table on the second page entries are made concerning the retention times of the individual peaks, the corresponding amounts and eventual peak names.

One-Point-Calibrations are performed in **Reprocess - Calculations** with the peak data of a calibration solution and the Calculation-File after entry of the corresponding level. The Report-File belonging to the chromatogram of a calibration solution contains for each peak the amount, the corresponding area and the response-factor.

A series of one-point calibrations can be carried out in **Reprocess, Calculations** via menu point ***Batch***. The mode is set to One Point Calibration, the standard chromatograms, the calculation file and the appropriate levels are entered into the Batch List. The calibrations are carried out on clicking OK.

Calculation of the calibration functions is carried out in the **Reprocess -Calculations** window by clicking ***Calibration*** and ***Multi-Calibration***. The Report-File obtained as described above is selected from the table. After clicking OK the approximation of the first order for the first peak appears on the screen in a graph showing the correlation between the concentration and peak area.

Select and ***Order*** allows you to display another approximation order. ***File*** and ***Save*** Results... stores the current approximation order displayed for all the peaks. Therefore enter a new name against Calculation File. The coefficients of the approximation functions are stored in the regression table on the third page of the calculation file as well as the retention times of the individual peaks. The peak table of the Calculation-File used to generate the first Report-File is taken over together with its retention times, amounts, response factors of the one-point calibration and the peak names. The parameter Regression for Calculation on the first page (method table) of the new file is automatically marked so that the approximation functions are used in later calculations instead of the response factors of the peak table.

5.3.2 Quantitative Calculation Methods

ChromStar offers six different quantitative calculation methods:

1) Percent method
2) Normalization method
3) Employing an external standard
4) Employing an internal standard
5) Employing a calibration function obtained by an external standard
6) Employing calibration functions for external and internal standards

These methods are to be defined by the corresponding entry in the Method field on the method table of the calculation file and also by the parameter Regression for Calculation and by identifying the internal standard peak in the peak table.

In quantitative calculations with the first three methods the universal formula applies:

$$RES_i = (PKS_i * RF_i) * MULT / DIV \quad (1)$$

in which

RESi = The result of the quantitative calculation for the i-th peak
PKSi = The peak area or height of the i-th peak
RFi = The response factor of the component i
(RF-value which is determined by the method of calibration)

MULT and DIV depend on the method employed and are described in the next sections.

5.3.2.1 Percent Method

In the percent method a percent calculation is performed of all peaks found. The factors of the formula (1) have the following values:

$RF_i = 1$
MULT = 100
DIV = ΣPKS_i

The formula now reads

$$RES_i = PKS_i * 1 * 100 / (\Sigma PKS_i)$$

No entries are required for this method on pages 2 and 3 of the Calculation-File. All peaks are calculated whose areas or heights are greater than the value against Minimum area/height in the method table of the Calculation-File. Carrying out the calculation with stored data is done in **Reprocess – Calculations,** ***Calculation*** by clicking the Report-File and the Calculation-File.

5.3.2.2 Normalization

In this method only those peaks are calculated which are defined in the peak table on page 2 of the Calculation-File and which are within a defined time window. The RF-value (response factor) is defined for each peak on page 2 of the Calculation-File. The default value is 1. This can be manually adjusted for each peak or defined in the calibration run. The other factors have the values:

DIV = $\Sigma\ (PKS_i * RF_i)$

MULT = 100

RF_i = amount/area of the i-th peak, defined in the calibration run

The formula (1) now reads

$$RES_i = \frac{PKS_i * RF_i * 100}{\Sigma\ (PKS_i * RF_i)}$$

5.3.2.3 External Standard Method

For quantitative calculations according to this method (Calculation-File with Method = External Standard) the RF-values (response factor) of the substances to be analysed are determined first in a calibration run (cp. 4.4.2.1) with a sample containing known amounts (Standard). Only those peaks are calculated which are within the defined time windows on page 2 of the calculation file. The factors of the formula (1) have the following values:

RF_i = amount/area of the component i,
defined in the calibration run for each peak

MULT = 1

DIV = 1

The formula (1) now reads:

$RES_i = PKS_i * RF_i$

In a current run the entry against Factor in Sample-Table of the method file is directly the value of the multiplier MULT in (1). This multiplier keeps the value 1 if no entry is made. If Weight in the sample table of the method file has a value other than 0, the proportional weight of each component of the solution compared to the total amount of the sample is calculated according to formula (2):

$RES_{i2} = RES_i * 100 / Wspl$ (2)

in which Wspl is the total amount of the sample as entered against Weight

The entry Recovery=Yes in the CHRST32.INI file in the section [Calibration] enables a quantitative evaluation under respect of a recovery rate. The calculation method should be External Standard. The recovery rate is entered in the calculation file in the Peak Table under Recovery (%) in %. This can be different for the different peaks. The default value is 100 for a recovery of 100%.

For the calculation of the amount the following formula is used:

$RES_i = PKS_i * RF_i * 100 / RC_i$

Wherin is:

RES_i = Result of the quantitative evaluation for the component i
PKS_i = Peak area or peak height of the ith peak (component i)
RF_i = Response factor of the component i
RC_i = Recovery rate in % for the component i

5.3.2.4 Internal Standard Method

In this method, which does not depend on an exact volume of the injection sample, a known quantity of a substance (internal standard) is added both to the calibration standard with known quantities and to the sample to be analysed.

The calculation which follows is based on this internal standard.

It is necessary in this method, to define the Standard-Peak as internal standard with IS against Code in the calculation file (Method = Internal Standard). If this definition is not found the following message appears:

"No internal standard peak defined"

The largest peak found in the time window defined with IS will be appointed as the Standard-Peak. If no peak is found within this time window the following error message is printed:

"No internal standard peak found"

Each of these two errors leads to a calculation according to the percent method

In calculations according to the Internal-Standard method the response factors are determined as follows:

$\text{Resp. Fact.}_{i} = \text{Amount}_{i\ Std.} * \text{PKS}_{IS\ Std.} / \text{PKS}_{i\ Std.}$

$\text{Resp. Fact.}_{IS} = \text{Amount}_{IS\ Std.} / \text{PKS}_{IS\ Std.}$

in which is:

$\text{Amount}_{i\ Std.}$ = Amount of the substance i in the standard solution

$\text{Amount}_{IS\ Std.}$ = Amount of the internal standard in the standardlsolution

$\text{PKS}_{IS\ Std.}$ = peak area or height of the internalstandard in the calibration run

$\text{PKS}_{i\ Std.}$ = peak area or height of the substance i in the calibration run

The amount of the sample is calculated according to:

$Amount_i$ = Resp. $Fact_i$ * PKS_i / PKS_{IS}

$Amount_{IS}$ = Resp. $Fact._{IS}$ * PKS_{IS} * $Menge_{IS\ Std.}$/$Menge_{IS\ Inj.}$

in which is:

PKS_i = peak area or height of the substance i

PKS_{IS} = peak area or height of the internal standard

$Amount_{IS\ Inj.}$ = Amount of the standard in the current run (defined in page 2 of the calculation file against Amt.in Std. or against Int.Std. before calculation in the box Update Input Values if an amount other than that in the standard solution is present)

In a current run the value of Amt. in Std. in the calculation file for the internal standard will be overwritten by the value of Int. Std. in the sample table of the method file if this has a value other than 0.

The result list of the quantitative calculation with Method = Internal Standard contains also the ratio between amount of the determined substance and amount of the Internal-Standard (Rel.).

For the internal standard is:

Rel = $amount_{IS}$ / $amount_{IS\ Inj.}$

5.3.2.5 External Standard Method with Calibration function

The calculation of the unknown quantities is made according to the equation

$$RES_i = K0_i + K1_i*PKS_i + K2_i*PKS_i^2 + K3_i*PKS_i^3 \quad (4)$$

in which is:

RES_i = result of the quantitative evaluation of the i-th peak

$K0_i$, $K1_i$, $K2_i$, $K3_i$ = regression coefficients of the 1st, 2nd and 3rd order for the component i

PKS_i = area or height of the i-th peak

The coefficients are determined by means of a multi-level calibration.

Herewith is also decided which order is employed for the calculation. Depending on the order selected K0 through K3 differ. The coefficients are stored in the regression table on the 3rd page of the calculation file. The Regression for Calculation parameter on page 1 must be marked. The peaks on page 2, for which also the multi-level calibration was performed, are calculated.

5.3.2.6 Internal Standard Method with Calibration function

In the Multi-Level Calibration with internal standard for each substance a regression function is calculated the dependency of the amount in the standard solution on the ratio $area_{Peak}$ to $area_{IS}$.

The calculation of the unknown quantities is made according to the formula:

$$\text{Amount}_i = K0_i + K1_i * (PKS_i / PKS_{IS}) + K2_i * (PKS_i / PKS_{IS})^2 + K3_i * (PKS_i / PKS_{IS})^3$$

in which

$K0_i, K1_i, K2_i, K3_i$ = regression coefficients of the 1st, 2nd and 3rd order for the component i

PKS_i = area or height of the ith peak

PKS_{IS} = area or height of the internal standard in the actual run

If the amount of the internal standard is different in the single standard solutions, as here it is, the area of the internal standard is normalized to the amount of the internal standard in the first calibration solution.

$$PKS_{IS\ Norm.} = PKS_{IS} * \text{amount}_{IS\ 1} / \text{amount}_{IS}$$

The coefficients are determined by means of a multi-level calibration. In this method the internal standard must be present in the standard solutions in varying quantities. When calibrating takes place the internal standard does not yet have to be defined. During the calibration is decided also which order will be used for evaluation. Depending on the order selected K0 through K3 differ. The coefficients are stored in the regression table on the 3rd page of the calculation file.

On page 2 the standard peak must be defined by IS against Code as the internal standard when the analysis run takes place. It must also be present in a known quantity in the solution to be analysed. This value is entered in the Update Input Values box against Int. Std. at the start of the calculation if the amount of the internal standard in the solution to be analysed is different to that of the first standard solution. Calculated are the peaks on page 2 (peak table) for which also the multi-level calibration has been performed.

5.3.3 Simple Quantitative Calculation

The quantitative calculation is performed in a number of steps:

1. Creating the necessary files (Method-,Data-Handling- and LC-Procedure-File).

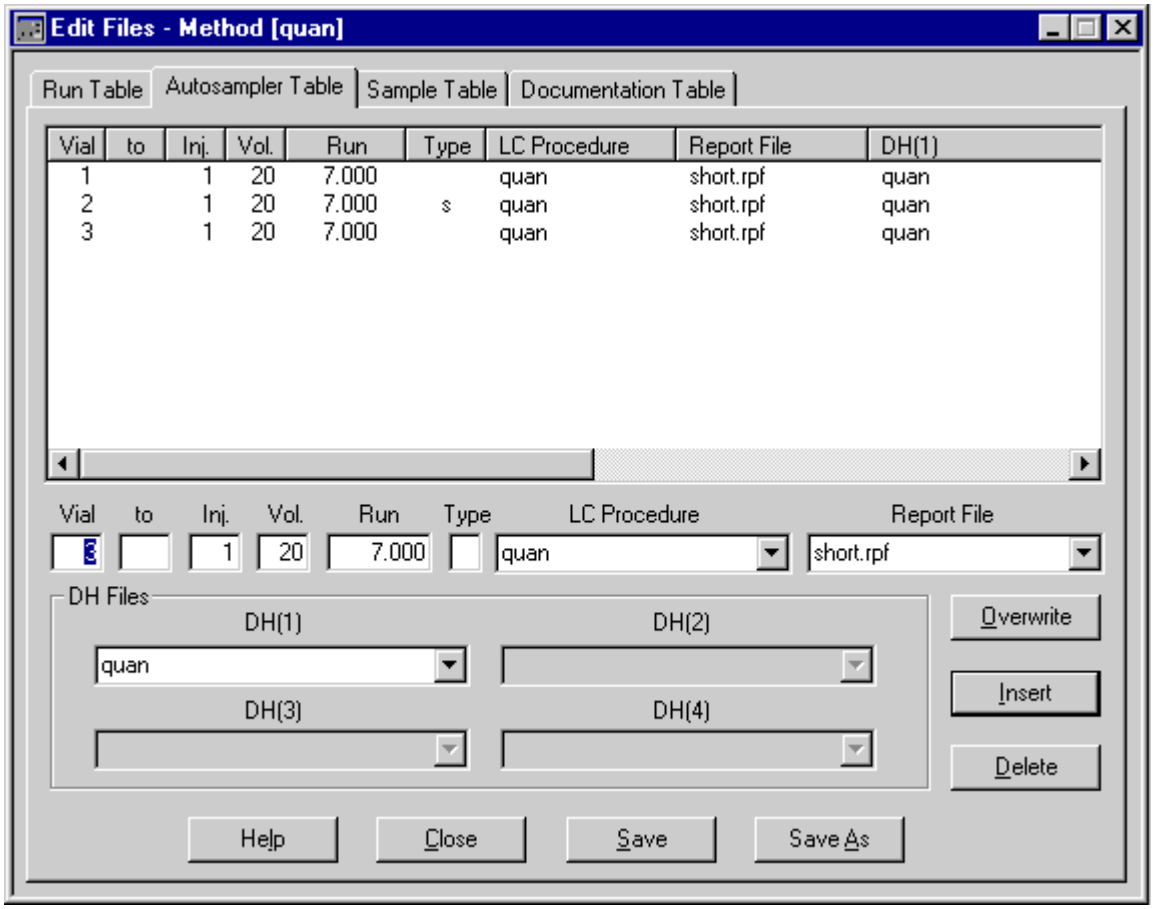
2. Determination of the retention times of the components contained in the calibration solution by recording a chromatogram (line 1 in the Autosampler-Table of the Method-File QUAN).

3. Creating the Calculation-File QUAN.

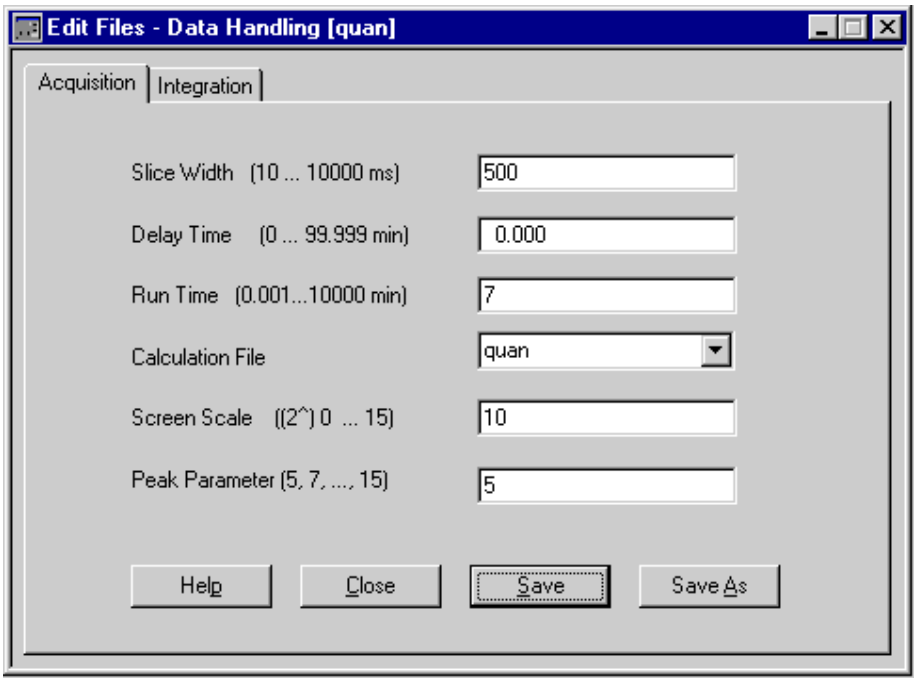
4. Determination of the response factors after recording a chromatogram of the calibration sample, in order to calculate amounts out of peak areas (one-point-calibration, line 2 in the autosampler table).

5. Determination of the unknown amounts after recording chromatograms of the sample (line 3 in the autosampler table).

The Method-File QUAN, created in Edit Files, contains on the first page only one entry, which is the name of the chromatogram to be recorded (in this case as first characters QUAN), also the Autosampler is marked and the print out Short Report is selected.



The Autosampler-Table contains the entries necessary to carry out the steps described before. The run time is 7 minutes. The solvent entries for the pump are defined in the LC-Procedure QUAN. Also the Data-Handling-File QUAN has to be used.

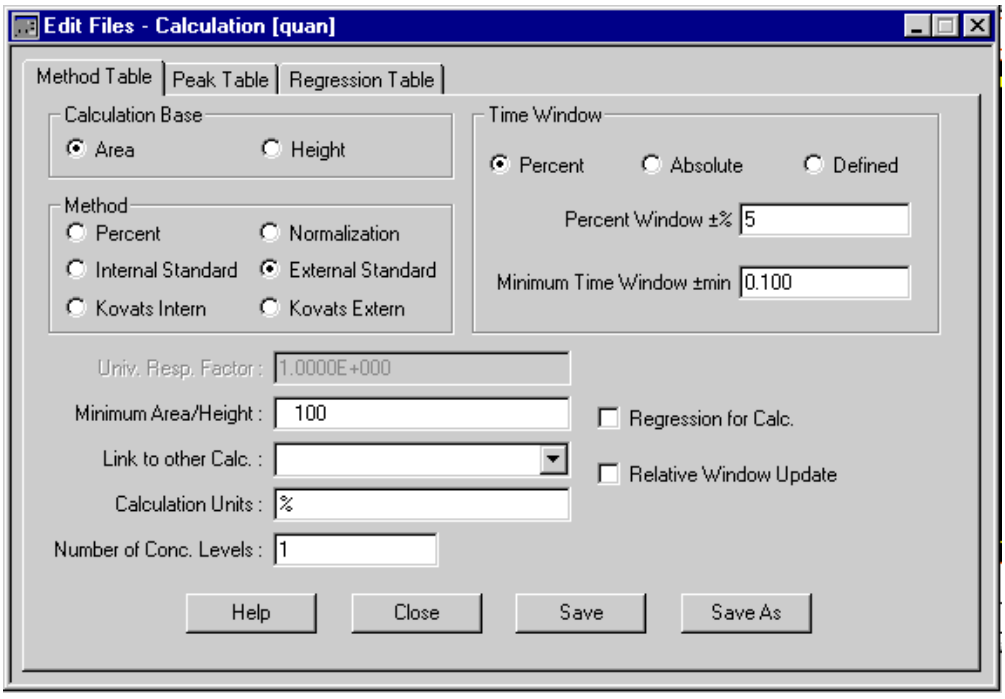


The Data-Handling-File QUAN contains the integration parameters and the name of the Calculation-File to be used.

Once these files (except the Calculation-File) have been set up and saved the method QUAN can be started from the point ***Analysis, Chromatogram*** in the **ChromStar**-window.

First the chromatogram (here QUAN0011) is recorded in order to determine the retention times. It is not important for the evaluation of the first chromatogram (QUAN0011) that the Calculation-File does not yet exist, a percent method will be performed.

Following upon this the Calculation-File QUAN is created.



In the first field on the first page of the Calculation-file QUAN is defined whether peak areas or peak heights are to be used as calculation basis. The method is defined in the second field, in this case External Standard. Minimum area/height and Link to other Calc. remain unchanged. The unit of concentration is entered against Calculation units, in this case %.

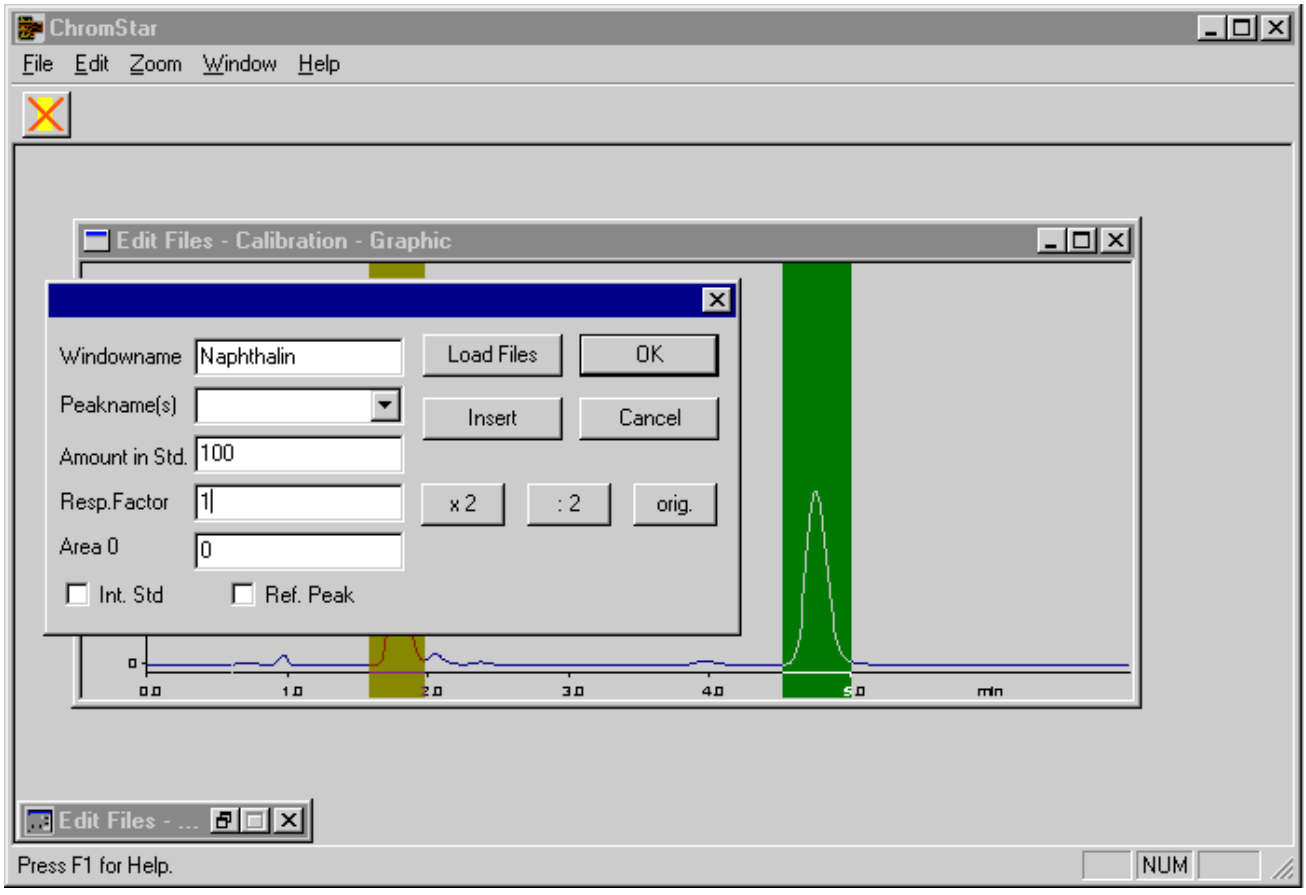
The other parameters remain unchanged.

The retention time and the amount for the substance to be determined (against Amt. in Std.) are entered on the peak table. The retention times of the chromatogram recorded first can be inserted in the peak table in different ways.

1. This is done by clicking the point ***Import Peaks...*** under ***File*** and selecting the corresponding file (QUAN0011) from the list of Report-Files.

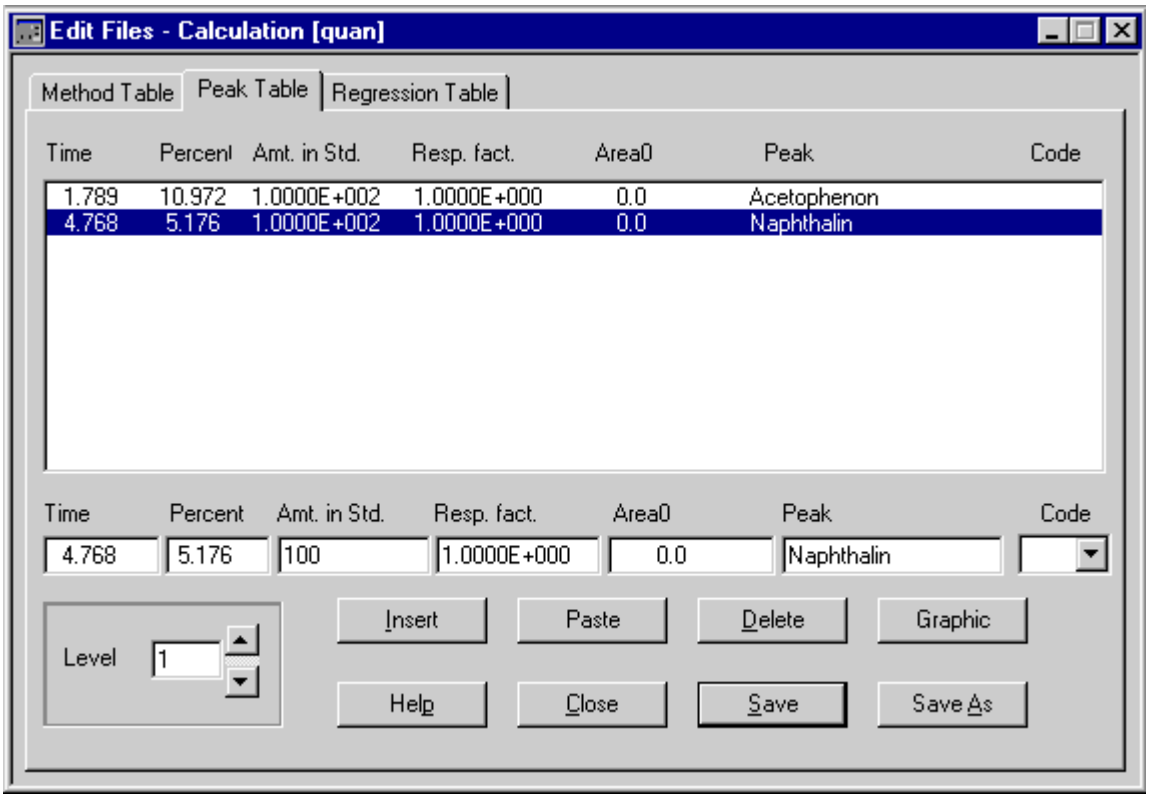
2. The same is done by reconstructing the chromatogram in ***Reprocess, Reconstruction,*** clicking ***Results***, marking the appropriate peaks and pressing the *Copy* key. The marked peaks are copied into the clipbaord. They now can be entered into the peak table of the Calculation file by using the *Paste* key.

3. Click into Grafic, select the chromatogram QUAN0011 by double click, drag the mouse with left key pressed over the peaks. Standard amounts and peak names can also be entered in this box. Quit with OK.



The values of Resp.fact. and Factor remain 1. The name of the component can be entered against Peak. The values of *Resp.fact*. and *Area0* remain unchanged.

The Calculation-File QUAN is saved.



The analysis is continued in the **Analysis** window. The method QUAN does not have to be recalled again with ***Method..., Load*** before the next injection. If it is recalled anyway a 2 must be entered against Start line. The new response factors are automatically transferred into the Calculation-File after recording the chromatogram of the calibration sample (here QUAN0021).

They also appear on the print out of the result list **19**).

By using the obtained response factors the amounts are calculated and printed immediately after recording the chromatogram (QUAN0031, **20**) of an injection of a mixture with unknown amounts of the substances used for the calibration.

At choice the quantitative evaluation of a chromatogram can also be performed later (cp. section 4.5.1).

5.3.4 Calculation Examples with Formulas

The following sections contain examples and formulas of calibrations and calculations. Arbitrary standard amounts were used in the calibrations. The quantitative results are mere fiction. Therefore the calculations 5.3.4.3 B and 5.3.4.3 C show different results for the same chromatogram.

5.3.4.1 Percent Method

A) with only a Data Handling file

Calculation:

Chromatogram : C:\CHRSTAR\DATA\BENA1111
Sample Identifier : B+T
Data Handling file : BENA *no name of a Calculation file is entered*
Calculation file :

Peak-No.	Ret.Time (min)	Pk.Start (min)	Pk.End (min)	Area	Height (mV)	Area%
1	0.600	0.283	0.833	904605	159.17	46.68
2	0.917	0.833	1.108	41248	9.49	2.13
3	1.750	1.658	1.908	429783	203.89	22.18
4	2.042	1.908	2.217	562232	249.50	29.01
Totals:				1937868	622.05	100.00

Formula:

$$RES_i = PKS_i * 1 * 100 / (\Sigma PKS_i)$$

Wherein is:

RES_i = Result of the quantitave evaluation for the i^{th} peak (Area%)

PKS_i = Peak area or height of the i^{th} peak

B) with a Calculation File (Method = Percent)

Calculation:

Chromatogram : C:\CHRSTAR\DATA\BENA1111
Sample Identifier : B+T
Data Handling file : BENA *with Calculation file BENA*
Calculation file : BENA *with Method = Percent*

Peak-No.	Win-No.	Ret.Time (min)	Area	Resp.-Fact.	Area%	Name
1		0.600	904605	1.000E+000	46.68	
2		0.917	41248	1.000E+000	2.13	
3	1	1.750	429783	1.000E+000	22.18	Benzene
4	2	2.042	562232	1.000E+000	29.01	Toluene
Totals:			1937868		100.00	

Formula:

$RES_i = PKS_i * 1 * 100 / (\Sigma PKS_i)$

Wherein is:

RES_i = Result of the quantitative evaluation for the i^{th} peak (Area%)

PKS_i = Peak area or height of the i^{th} peak

5.3.4.2 Normalization Method

Calculation:

Chromatogram : C:\CHRSTAR\DATA\BENA1111
Sample Identifier : B+T
Data Handling file : BENA *witht Calculation file BENANORM*
Calculation file : BENANORM *witht Method = Normalization*

Peak-No.	Win-No.	Ret.Time (min)	Area	Resp.-Fact.	Area%	Name
3	1	1.750	429783	1.000E+000	43.32	Benzene
4	2	2.042	562232	1.000E+000	56.68	Toluene
Totals:			992014		100.00	

Formula:

$$RES_i = \frac{PKS_i * RF_i * 100}{\Sigma (PKS_i * RF_i)}$$

Wherein is:

RES_i = Result of the quantitative evaluation for the i^{th} peak (Area%)

PKS_i = Peak area or height of the i^{th} peak

In the Normalization method only peaks mentioned in the Peak table of the Calculation file are used for the calculation.

5.3.4.3 External Standard Method

A) after one-point calibration

Ein-Punkt-Kalibration:

Chromatogram : BENA2111 *Chromatogram of the standard solution*
Sample Identifier : B+T
Calculation File : BENAEXT
Calculation Method: Calibration

Peak-No.	Win-No.	Ret.Time (min)	Area	Resp. Fact.	Std.Amt. (AU *)	Name
1	1	1.758	794832	1.258E-004	1.000E+002	Benzene
2	2	2.033	1096267	1.368E-004	1.500E+002	Toluene
Totals:			1891099			

** Amount in the standard solution*

Formula:

RF_i = Amount/area of the component i,
is calculated in the calibration run for each peak

Calculation of the unknown amounts:

Chromatogram : BENA3111 *Chromatogram of the mixture with unknown amounts*
Sample Identifier : B+T
Calculation File : BENAEXT
Calculation Method: External Standard
Calibration Method : One Point

Peak-No.	Win-No.	Ret.Time (min)	Area	Resp.-Fact.	Absolute (AU)	Weight (%)	Name
1	1	1.750	1115697	1.258E-004	1.40E+002	0.00	Benzene
2	2	2.042	1577773	1.368E-004	2.16E+002	0.00	Toluene
Totals:			2693471		3.56E+002	0.00	

Formula:

$RES_i = PKS_i * RF_i$

Wherein is:

RES_i = Result of the quantitative evaluation for the i^{th} peak (Absolute)

PKS_i = Peak area or height of the i^{th} peak

RF_i = Standard amount/area of the component i,
is calculated for each peak in the calibration run

B) after calibration with averaging the peak areas

Calibration with average calculation

Nr.	Result-File-Name	*Chromatograms of the standard solutions*
1	BENA1111	*The same standard solution is injected twice.*
2	BENA6121	

Peak Name: Benzene Time Window: 1.75

Std.Amt	Area
1.0000E+002	429783
1.0000E+002	420346

Regression Analysis

Average Area = 4.2506E+005
Standard Deviation = +-6.6725E+003 (+- 1.6%)
Confidence Region = +-5.9968E+004
K1 = 2.3526E-004

Peak Name: Toluene Time Window: 2.06

Std.Amt	Area
1.5000E+002	562232
1.5000E+002	550833

Regression Analysis

Average Area = 5.5653E+005
Standard Deviation = +-8.0600E+003 (+- 1.4%)
Confidence Region = +-7.2438E+004
K1 = 2.6953E-004

Formula:

Average Area i = Σ (1 to n) (Area i) / n

n = number of chromatograms
i = number of components in the standard solution
K1i = Amount of the standard i / (Average Area i)

The averaged response factors are entered in the Regression Table of the calculation file BENAEXT.CAL as K1, the parameter *Regression for Calculation* is automatically ticked.

Calculation of the unknown amounts:

Chromatogram : BENA9121 *Chromatogram of the sample with unknown amounts*
Sample Identifier : B+T *of the solutes*
Calculation File : BENAEXT
Calculation Method: External Standard
Calibration Method : Multi Level

Peak-No.	Win-No.	Ret.Time (min)	Area	Resp.-Fact.	Absolute (AU)	Weight (%)	Name
1	1	1.758	1358186	2.353E-004	3.20E+002	0.00	Benzene
2	2	2.050	1990622	2.695E-004	5.37E+002	0.00	Toluene
Totals:			3348808		8.56E+002	0.00	

Formula:

$RES_i = PKS_i * K1_i$

Wherein is:

RES_i = Result of the quantitative evaluation for the i^{th} peak (Absolute)
PKS_i = Peak area or height of the i^{th} peak
$K1_i$ = Standard amount/average area of the component i, is calculated for each peak in the calibration run

C) after Calibration with regression function (Multi Level Calibration)

Multi Level Calibration

Nr.	Result-File-Name
1	bena1111
2	bena2111
3	bena3111
4	bena4111
5	bena5111

Chromatograms of the standad solutions

5 Standard solutions with 5 different amounts are injected.

Peak Table

Peak Name: Benzene Time Window: 1.77

```
Std.Amt              Area
========================================
1.0000E+000       429783
2.0000E+000       794832
3.0000E+000      1115697
4.0000E+000      1380790
5.0000E+000      1548442
----------------------------------------------------------------------
Regression Analysis
-------------------
K0 = 4.5045E-001          Coeff. of Correlation  = 0.998627
K1 = 7.6158E-007          Std. Error of Estimat. = 0.082819
K2 = 1.3722E-012
K3 = 0.0000E+000
----------------------------------------------------------------------

Peak Name: Toluene                Time Window:   2.07
Std.Amt              Area
========================================
1.0000E+000       562232
2.0000E+000      1096267
3.0000E+000      1577773
4.0000E+000      2014605
5.0000E+000      2410053
----------------------------------------------------------------------
Regression Analysis
-------------------
K0 = 1.0376E-001          Coeff. of Correlation  = 0.999994
K1 = 1.4682E-006          Std. Error of Estimat. = 0.005601
K2 = 2.3303E-013
K3 = 0.0000E+000
----------------------------------------------------------------------
```

The regression function of 2nd order is calculated.
The regression koefficients are saved as K0, K1 and K2 in the Regression Table of the calculation file BENAML.CAL, in the Method Table the parameter *Regression for Calculation* ia automatically ticked.

Calculation of the unknown amounts:

```
Chromatogram : BENA9121          Chromatogram of the sample mixture
Sample Identifier : B+T                with unknown amounts
Calculation File  : BENAML
Calculation Method: External Standard
Calibration Method : Multi Calibration (Regression  of the 2nd Order)


Peak-  Win-  Ret.Time   Area          Resp.-    Absolute   Weight    Name
 No.   No.   (min)                    Fact.     ( AU )       (%)
-------------------------------------------------------------------------------------
  1     1      1.758    1358186      --------   4.02E+000  0.00     Benzene
```

2	2	2.050	1990622	--------	3.95E+000	0.00	Toluene
Totals:			3348808		7.97E+000	0.00	

Formula:
The calculation of the unknown amounts (Absolute) is carried out according to the equation:

$$RES_i = K0_i + K1_i*PKS_i + K2_i*PKS_i^2 + K3_i*PKS_i^3$$

where

RES_i = Result of the quant. Evaluation for the i^{th} peak (Absolute)

$K0_i$, $K1_i$, $K2_i$, $K3_i$ = Regression koefficients of the 1^{st}, 2^{nd} or 3^{rd} Ordnung for the component i

PKS_i = peak area or height of the i^{th} peak

The coefficients are calculated during multi-level-calibration.

5.3.4.4 Internal Standard Method

A) After one-point calibration

One-point calibration:

Chromatogram : BENA6121 *Chromatogram of the standard solution,*
Sample Identifier : B+T *Toluene is the internal Standard =IS*
Calculation File : BENAIS
Calculation Method: Calibration

Peak-No.	Win-No.	Ret.Time (min)	Area	Resp. Fact.	Std.Amt. (AU *)	Name
1	1	1.775	420346	2.621E+001	2.000E+001	Benzene
2	2 (IS)	2.075	550833	3.631E-005	2.000E+001	Toluene
Totals:			971179			

** Std.Amt. =Amount in the standard solution*

Formula:

$$\text{Resp. Fact.} = \text{amount}_{\text{Peak Std.}} * \text{area}_{\text{IS Std.}} / \text{area}_{\text{Peak Std.}}$$

For the internal standard the Resp. Fact. is calculated according to:

$$\text{Resp. Fact.}_{\text{IS}} = \text{amount}_{\text{IS Std.}} / \text{area}_{\text{IS Std.}}$$

Calculation of the unknown amounts:

Chromatogram : BENA1111 *Chromatogram of the mixture with unknown amounts*
Sample Identifier : B+T

Calculation File : BENAIS
Calculation Method: Internal Standard
Calibration Method : One Point

Peak-No.	Win-No.	Ret.Time (min)	Area	Resp.-Fact.	Absolute (AU)	Rel	Weight (%)	Name
3	1	1.750	429783	2.62E+001	2.003E+001	1.00	0.0	Benzene
4(IS)	2	2.042	562232	3.63E-005	2.041E+001	1.02	0.0	Toluene
Totals:			992014		2.003E+001		0.0	

Formula:
The amounts under *Absolute* are calculated according to the formulas:

$\text{Amount}_{\text{Peak}} = \text{Resp.Fact.}_{\text{Peak}} * \text{area}_{\text{Peak}} / \text{area}_{\text{IS}}$

$\text{Amount}_{\text{IS}} = \text{Resp.Fact.}_{\text{IS}} * \text{area}_{\text{IS}} * \text{amount}_{\text{IS Std.}} / \text{amount}_{\text{IS Inj.}}$

If another amount of internal standard is injected in the sample mixture than in the calibration mixture, the area_{IS} is multiplied with the ratio $\text{amount}_{\text{IS Std.}} / \text{amount}_{\text{IS Inj.}}$
In this example the amount of internal standard is in the standard solution($\text{amount}_{\text{IS Std.}}$) and in the solution with unknown amounts ($\text{amount}_{\text{IS Inj.}}$) the same (= 20)
For the internal standard the ratio *Rel*. of calculated amount to injected amount is calculated.

$\text{Rel.} = \text{amount}_{\text{IS}} / \text{amount}_{\text{IS Inj.}}$

B) after calibration with average values

Calibration with averaging

Calculation Mode: Internal Standard

Nr.	Result-File-Name	*Chromatograms of the standard solution*
1	bena1111	*The same standard solution is injected twice,*
2	bena6121	*Toluene is the internal standard =IS*

Peak Table

Peak Name: Benzene Time Window: 1.75

Std.Amt	Area/Area(IS)	Area
2.0000E+001	0.764	429783
2.0000E+001	0.763	420346

Regression Analysis

Average Area/Area(IS) = 7.6377E-001
Standard Deviation = +-9.2812E-004 (+- 0.1%)

```
Confidence Region      = +-8.3413E-003
K1 = 2.6186E+001
-----------------------------------------------------------------

Peak Name: Toluene                Time Window:   2.06
Std.Amt        Area/Area(IS)       Area
=================================================
2.0000E+001          ----          562323
2.0000E+001          ----          550833
-----------------------------------------------------------------
Regression Analysis
-------------------
K0 = 0.0000E+000          Coeff. of Correlation  = 0.000000
K1 = 1.0000E+000          Std. Error of Estimat. = 0.000000
K2 = 0.0000E+000
K3 = 0.0000E+000
-----------------------------------------------------------------
```

Formula:

Average Area/Area(IS) i = Σ (1 bis n) (Area i /Area(IS)) / n
n = number of chromatograms
i = number of components in the standard solution
K1i = amount of the standard i / (Average Area/Area(IS) i)

The averaged response factors are saved as K1 in the Regression Table of the calculation file BENAISMP.CAL, in the Method Table the parameter *Regression for Calculation* is automatically ticked.
For the internal standard no average calculation is carried out.

Calculation of the unknown amounts:

Chromatogram : BENA7121 *Chromatogram of the mixture with unknown amounts*
Sample Identifier : B+T
Calculation File : BENAISMP
Calculation Method: Internal Standard
Calibration Method : Multi Level
Internal Standard: **40.00**
In the sample solution the internal standard was added in the double amount!

Peak-No.	Win-No.	Ret.Time (min)	Area	Resp.-Fact.	Absolute (AU)	Rel (%)	Weight	Name
1	1	1.758	792307	2.62E+001	3.819E+001	0.95	0.0	Benzene
2(IS)	2	2.050	1086671	---------	3.866E+001	0.97	0.0	Toluene
Totals:			1878978		3.819E+001	0.0		

Formula:
The calculation of the amount under *Absolute* is carried out according to the formulas:

amount $_{Peak}$ = K1 $_{Peak}$ * area $_{Peak}$ / area $_{IS}$

amount $_{IS}$ = Resp.Fact. $_{IS}$ * area $_{IS}$ * amount $_{IS\ Std.}$ / amount $_{IS\ Inj.}$

If another amount of internal standard than in the standard solution is injected, the area $_{IS}$ is multiplied by the ratio area $_{IS\ Std.}$ / area $_{IS\ Inj.}$

In the example the standard solution contains 20AU internal standard, the sample solution contains 40AU internal standard.

For the internal standard the ratio *Rel*. of calculated amount to injected amount is calculated.

Rel. = amount $_{IS}$ / amount $_{IS\ Inj.}$

C) after calibration with regression functionfunktion (Multi Level Calibration)

Multi Level Calibration

Calculation Mode: Internal Standard

Nr.	Result-File-Name	*Chromatograms of the standard solutions*
1	bena1111	*5 standard solutions with 5 different amounts*
2	bena2111	*are injected*.
3	bena3111	*Toluene is the internal standard =IS*
4	bena4111	
5	bena5111	

Peak Table

Peak Name: Benzene Time Window: 1.77

Std.Amt	Area/Area(IS)
1.0000E+000	0.764
2.0000E+000	1.450
3.0000E+000	2.121
4.0000E+000	2.742
5.0000E+000	3.212

Regression Analysis

K0 = -3.1011E-001 Coeff. of Correlation = 0.997609

K1 = 1.6084E+000 Std. Error of Estimat. = 0.109281

K2 = 0.0000E+000

K3 = 0.0000E+000

Peak Name: Toluene Time Window: 2.07

Std.Amt	Area	*normalized area*
1.0000E+000	562232	562232

2.0000E+000	1096267	548133
3.0000E+000	1577773	525924
4.0000E+000	2014605	503651
5.0000E+000	2410053	482010

For benzene the regression function of the 1st order for the dependency of the amount in the standard solution on the ratio $area_{Peak}$ to $area_{IS}$ is calculated.
The regression coefficients are saved in the Regression Table of the calculation file BENAISML.CAL as K0 and K1. In the Method Table the parameter *Regression for Calculation* is automatically ticked.
Toluene is the internal standard, no regression function is calculated for this.

If the amount of the internal standard is different in the single standard solutions, as here it is, the area of the internal standard is normalized to the amount of the internal standard in the first calibration solution.

$$PKS_{IS\ Norm.} = PKS_{IS} * amount_{IS\ 1} / amount_{IS}$$

Calculation of the unknown amounts:

Chromatogram : BENA8121 *Chromatogram of the mixture with unknown amounts*
Sample Identifier : B+T
Calculation File : BENAISML
Calculation Method: Internal Standard
Calibration Method : Linear Fit
Internal Standard: **3.00**
In the sample solution the internal standard was added in the three times amount!

Peak-No.	Win-No.	Ret.Time (min)	Area	Resp.-Fact.	Absolute (AU)	Rel	Weight (%)	Name
1	1	1.758	1102628	---------	3.104E+000	1.03	0.0	Benzene
2(IS)	2	2.050	1558244	---------	2.772E+000	0.92	0.0	Toluene
Totals:			2660872		3.104E+000		0.0	

Formula:

The unknown amounts are calculated according to:

$$Amount_i = K0_i + K1_i * (PKS_i / PKS_{IS}) + K2_i * (PKS_i / PKS_{IS})^2 + K3_i * (PKS_i / PKS_{IS})^3$$

wherein is

$K0_i, K1_i, K2_i, K3_i$ = Regression coefficients of the approximation of the 1st, 2nd or 3rd order for the component i

PKS_i = area or height of the i_{th} peak

PKS_{IS} = area or height of the internal standard in the actual run, in this case normalized.

6. Printer Protocols

This chapter contains a series of print-outs of chromatograms, results lists, calculations, calibration curves etc., obtainable when working with ChromStar. The bold printed numbers in the previous chapters refer to the figures in this part.

6.1 Print-outs in Edit Files

1 Print-out of a method file

Current user : H. Risler Date: 17.04.1996 Time: 11:30

Method File: C:\CHRSTNEU\DATA\NEU.MET
Created by: H. Risler on:03/21/96 at:11:31:08
Last Updated by: H. Risler on:04/17/96 at:11:30:06

Run-Table

Notes : Test CS40, AS, LC22

Report : short.rpf
Autosampler : Y
Normalisation : Y
Data File (1): NE00
Data File (2):
Data File (3):
Data File (4):

Autosampler-Table (Included - 3 Vials)

Vial	to	Inj.	Vol.	Delay	Run Type	LC-PROC	DH(1)	DH(2)	DH(3)	DH(4
1	5	1	20	0.00	4.000 s	neu	neu			
6		1	20	0.00	4.000	neu	neu			
7		1	20	0.00	4.000	neu	neu			

Sample-Table (Included - 7 Samples)

Vial	Sample Id.	Factor	Weight	Int.Std.	Conc.Level
1	S(3,6)-1-Std	1.000000	0.000000	0.000000	1
2	S(3,6)-2-Std	1.000000	0.000000	0.000000	2
3	S(3,6)-3-Std	1.000000	0.000000	0.000000	3
4	S(3,6)-4-Std	1.000000	0.000000	0.000000	4
5	S(3,6)-5-Std	1.000000	0.000000	0.000000	5
6	S(3,6)-Probe1	1.000000	0.000000	0.000000	1
7	S(3,6)-Probe2	1.000000	0.000000	0.000000	1

Method-file (cont.)

```
                                Page:  2       Date: 17.04.1996  Time: 11:30
Output of: C:\CHRSTNEU\DATA\NEU.MET

        Documentation-Table

   User Details

   Name : S(3,6)
   Origin : Alkylbenzole
   Preparation : in CH3CN/H2O
   Injection vol. : 20 µl


   Mobile Phase

   A    :
   B    :
   C    :
   D    : 85% CH3CN + 15 % H2O
   Notes: Flow 1ml/min


   Column Details

   Type  : RP18
   Size  : 125x4.5mm
   Particle size : 5 µm
   Notes :


   Detector Details

   Type            : UV
   Range           :
   Wavelength (nm) : 254
   Notes           :


End of Method File Report on 17.04.1996 at 11:30. Included 2 Pages.
```

2 Print-out of data handling file

```
Current user : H. Risler                    Date: 17.04.1996  Time: 11:31

Data Handling File: NEU2
            Created by: H. Risler                on:03/21/96 at:11:32:08
            Last Updated by: H. Risler           on:04/17/96 at:11:31:47

        Acquisition-Page

            Slice Width (msec)    :  500
            Delay Time (min)      :    0.000
            Run Time (min)        :    5.000
            Calculation File      :  NEU2
            Screen Scale (2^)     :   10
            Peak Parameter        :    5

        Integration-Page

            Time    Function          Value
              0.000 Noise       1000.000
              0.000 Integrate Inhibit   1.000
              3.000 Noise        300.000

End of Data Handling Report on 17.04.1996 at 11:31. Included 1 Page.
```

3 Print-out of a calculation file

Current user : H. Risler Date: 17.04.1996 Time: 11:34

Calculation File: QU4-ML
Created by: H. Risler on:12/14/95 at:09:39:55
Last Updated by: H. Risler on:12/22/95 at:08:43:28

Method-Table

Calculation base	:Area
Method	:External Standard
Minimum area/height	:100000
Link to other Calc.	:
Calculation Units	:
No. Concentration Level	: 5
Regression for Calc. (Y/N)	:Y
Relative Window Update (Y/N)	:N
Time Window	:Percent
Percent Window ±%	: 5
Minimum Time Window ±min.	: 0.100

Peak-Table

Time	+-Percent	Amt.in Std.	Resp.Fact.	Area0	Peak	Code
Level 1						
1.990	5.000	1.0000E+001	3.1254E-005	0	Toluol	
2.260	5.000	3.0000E+001	6.4538E-005	0	Ethylbenzol	
2.530	5.000	2.0000E+001	6.7848E-005	0	Cumol	
3.300	5.000	5.0000E+001	1.5072E-004	0	Butylbenzol	
5.500	5.000	1.0000E+002	4.3209E-004	0	Hexylbenzol	
Level 2		2.0000E+001				
		6.0000E+001				
		4.0000E+001				
		1.0000E+002				
		2.0000E+002				
Level 3		3.0000E+001				
		9.0000E+001				
		6.0000E+001				
		1.5000E+002				
		3.0000E+002				
Level 4		4.0000E+001				
		1.2000E+002				
		8.0000E+001				
		2.0000E+002				
		4.0000E+002				
Level 5		5.0000E+001				
		1.5000E+002				
		1.0000E+002				
		2.5000E+002				
		5.0000E+002				

Regression-Table

Time	K0	K1	K2	K3
1.990	8.8172E-001	3.2721E-005	0.0000E+000	0.0000E+000
2.260	-9.9585E+000	9.4171E-005	-9.9287E-012	0.0000E+000
2.530	-1.3344E+001	1.3113E-004	-7.2747E-011	2.4303E-017
3.300	1.3878E+001	1.5595E-004	0.0000E+000	0.0000E+000
5.500	3.6462E+001	4.1729E-004	0.0000E+000	0.0000E+000

End of Calculation Report on 17.04.1996 at 11:34. Included 2 Pages.

4 Print-out of an LC procedure file

Current user : H. Risler Date: 17.04.1996 Time: 11:35

LC-Procedure: GRADIENT
Created by: H. Risler on:01/29/96 at:08:56:52

Time	Function	Value			
0.00	Solvents	80.0	20.0	0.0	0.0
2.00	Solvents	80.0	20.0	0.0	0.0
4.00	Solvents	10.0	90.0	0.0	0.0
6.00	Solvents	10.0	90.0	0.0	0.0
16.00	Solvents	100.0	0.0	0.0	0.0
20.00	Solvents	100.0	0.0	0.0	0.0
22.00	Solvents	80.0	20.0	0.0	0.0
25.00	Solvents	80.0	20.0	0.0	0.0

End of LC Procedure Report on 17.04.1996 at 11:35. Included 1 Page.

5 Print-out of the preset table

Current user : H. Risler Date: 17.04.1996 Time: 11:36

PRESET SYSTEM PARAMETERS

Flow rate (ml/min)	:	1.00
Max press (bar)	:	400
Min press (bar)	:	0
Wavelength (nm)	:	254
Attenuate	:	10.000
Screen scale (2^)	:	10
Compress factor	:	0
Slice width (msec)	:	500
Noise	:	500
Threshold (mV)	:	500
Skim ratio	:	3
Syringe vol (ul)	:	250
Bubble check (Y/N)	:	N
Oven temperature	:	0
Rep. AS Start (Y/N)	:	N
Emission (nm)	:	20

6.2 Print-outs of chromatograms using the report print templates (.RPF)

6 Print-out after recording a chromatogram with SHORT.RPF

Chromatogram: AROM0001 Date: 14.03.1996 Time: 8:45

Author: H. Risler

Notes:

SCPA
SOFTWARE FÜR CHROMATOGRAPHIE UND PROZESS - ANALYTIK GMBH

Sample Identifier:

Injected on: 26.01.1996 Injected at: 13:28

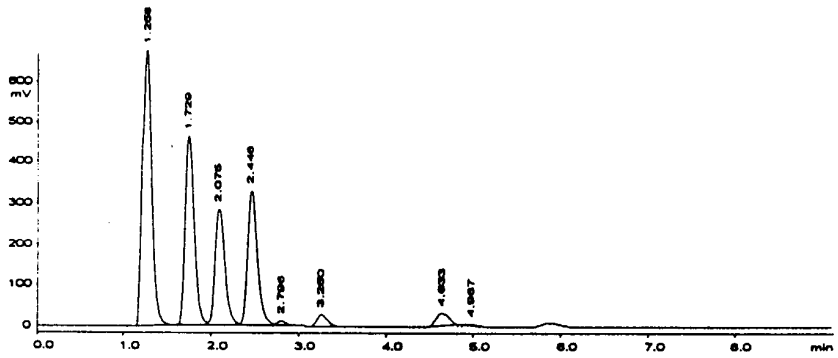
Data Processing Parameters

Slice Width (msec): 250 Noise: 500

Threshold (mV): 500 Skim Ratio: 3

Parameter Files

Data Handling File: AROMATEN Calculation-File: AROMATEN



Calculation Method : Percent

Peak-No	Ret.Time (min)	Pk.Start (min)	Pk.End (min)	Area	Height (mV)	Area%
1	1.258	1.138	1.538	2443481	677.39	36.639
2	1.729	1.612	1.950	1576444	463.47	23.638
3	2.075	1.950	2.317	1070014	284.88	16.045
4	2.446	2.317	2.917	1272040	328.02	19.074
5	2.796	2.700	2.917	33627	10.04	0.504
6	3.250	3.138	3.433	105701	26.74	1.585
7	4.633	4.463	4.821	156164	29.51	2.342
8	4.967	4.867	5.096	11537	2.88	0.173
				6669009	1822.94	100.000

Page: 1 of: 1

7 Print-out with LONG.RPF

The result list of the integration with Mode=HPLC/GC contains retention times, peak start and peak end time, peak areas, peak heights and area percent of the individual peaks. This evaluation is obtained when no calculation file has been defined in the data handling file, when the defined calculation file does not exist or when a calculation file is defined in which the parameters Area and Percent (s. 4.1.3.1) are marked.

Chromatogram: AROM0001 Date: 7.08.2001 Time: 12:34

Author: H. Risler

Notes:

Sample Identifier:

Injected on: 21.02.1994 Injected at: 14:20

Data Processing Parameters

Slice Width (msec): 250 Noise: 500

Threshold (mV): 500 Skim Ratio: 3

Parameter Files

Data Handling File: AROMATEN Calculation-File: AROMATEN

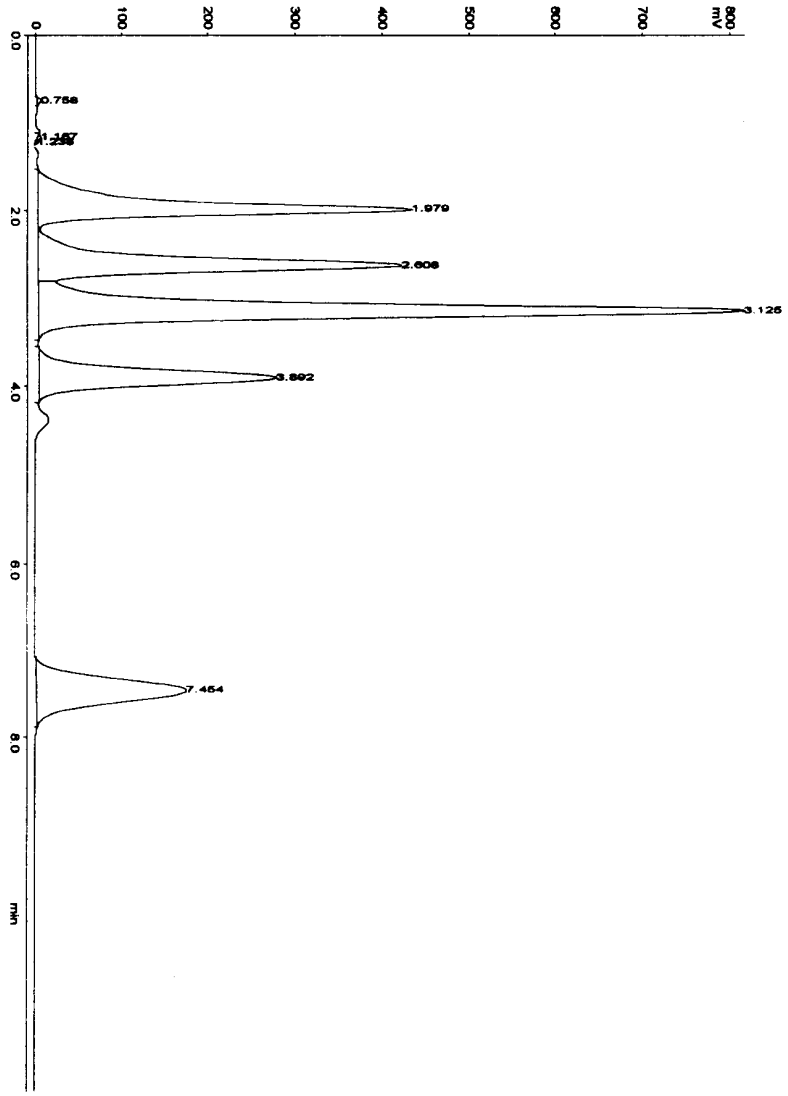
Calculation Method : Percent

Peak-No	Ret.Time (min)	Pk.Start (min)	Pk.End (min)	Area	Height (mV)	Area%
1	0.758	0.717	0.829	8925	5.27	0.073
2	1.167	1.129	1.275	5290	2.99	0.043
3	1.237	1.217	1.275	3585	3.61	0.029
4	1.979	1.529	2.213	2249781	431.73	18.325
5	2.608	2.213	2.804	2260352	419.81	18.411
6	3.125	2.804	3.467	4408528	813.23	35.909
7	3.892	3.550	4.183	1684454	274.23	13.721
8	7.454	7.071	7.883	1655972	173.84	13.489
				12276886	2124.71	100.000

Page: 1 of 2

7 (Cont.)

Chromatogram: AROM0001 Date: 7.08.2001 Time: 12:34



Page: 2 of 2

8 Print-out of the results list of a quantitative evaluation (calculation-file with *Method* = External Standard) using the report print template TABLE.RPL

Chromatogram: qu5-0012 Date: 7.08.2001 Time: 12:38

Author: H. Risler

Notes: Test CS 3.2 Quant.
Auswertung

Sample Identifier: Testmix5

Injected on: 14.12.1993 Injected at: 8:29

Data Processing Parameters

Slice Width (msec): 500 Noise: 100

Threshold (mV): 500 Skim Ratio: 3

Parameter Files

Data Handling File: QU1 Calculation-File: QU1-ML

Calculation Method : External Standard
Calibration Method : Multi Calibration

Peak-No	Win No.	Ret.Time (min)	Area	Resp.-Fact.	Absolute Arb.U.	Name
4	1	1.992	928661	3.1278E-05	31.361	Toluol
5	2	2.250	1288667	6.4635E-05	91.193	Ethylbenzol
6	3	2.517	853263	6.8070E-05	59.352	Cumol
8	4	3.292	896905	1.5173E-04	154.034	Butylbenzol
10	5	5.550	636728	4.2879E-04	302.848	Hexylbenzol
			4604224		638.788	

Page: 1 of 1

6.3 Print-outs of calibrations

9 Result list of a one-point-calibration

Chromatogram: qu4-0011 Date: 14.03.1996 Time: 9:05

Author: H. Risler

Notes: Test CS 3.2 Quant.
Auswertung

Sample Identifier: Testmix5
Injected on: 7.12.1993 Injected at: 9:29

Data Processing Parameters

Slice Width (msec): 500 Noise: 500
Threshold (mV): 500 Skim Ratio: 3

Parameter Files

Data Handling File: QUX Calculation-File: QÜ4

Calculation Method : Calibration

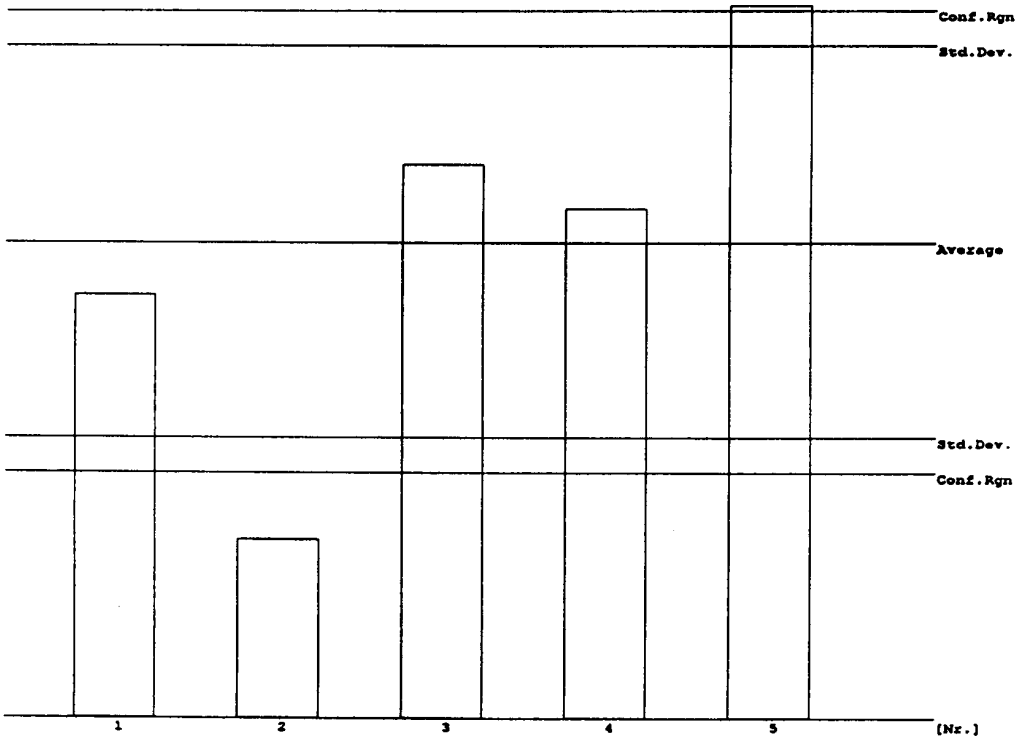
Peak-No	Win No.	Ret.Time (min)	Area	Resp.-Fact.	Std.Amt.	Name
3	1	2.008	319709	3.1278E-05	10.000	Toluol
4	2	2.308	464142	6.4635E-05	30.000	Ethylbenzol
5	3	2.600	293817	6.8070E-05	20.000	Cumol
6	4	3.417	329533	1.5173E-04	50.000	Butylbenzol
7	5	5.625	233212	4.2879E-04	100.000	Hexylbenzol
			1640412			

10 Result list of a multi-point-calibration
(here only shown for the first two peaks)

```
Multi Calibration Table        Date: 14.03.1996    Time:  9:06

Calculation Mode: External Standard

Standard Sample Table

Nr.  Report-File-Name
 1        qum10003
 2        qum10004
 3        qum10005
 4        qum10006
 5        qum10007


Peak Table
Peak Name: Toluol                          Time Window:   2.02
 Std.Amt          Area
==========================================================================
4.0000E+001       1148872
4.0000E+001       1137723
4.0000E+001       1154783
4.0000E+001       1152789
4.0000E+001       1162010
--------------------------------------------------------------------------
Regression Analysis
Average Area             =   1.1512E+006
Standard Deviation       = +-8.9339E+003 ( +-  0.8% )
Confidence Region        = +-1.0542E+004

K0 = 0.0000E+000
K1 = 3.4745E-005
--------------------------------------------------------------------------

Peak Name: Ethylbenzol                     Time Window:   2.30
 Std.Amt          Area
==========================================================================
1.2000E+002       1650572
1.2000E+002       1650355
1.2000E+002       1671387
1.2000E+002       1670598
1.2000E+002       1681258
--------------------------------------------------------------------------
Regression Analysis
Average Area             =   1.6648E+006
Standard Deviation       = +-1.3774E+004 ( +-  0.8% )
Confidence Region        = +-1.9084E+004

K0 = 0.0000E+000
K1 = 7.2079E-005
--------------------------------------------------------------------------
```

11 Graphical presentation of a multi-point-calibration
(here only shown for the first peak)

Multi Point Calibration Date: 14.03.1996 Time: 9:06
Peak Name : Toluol
Time Window : 2.02



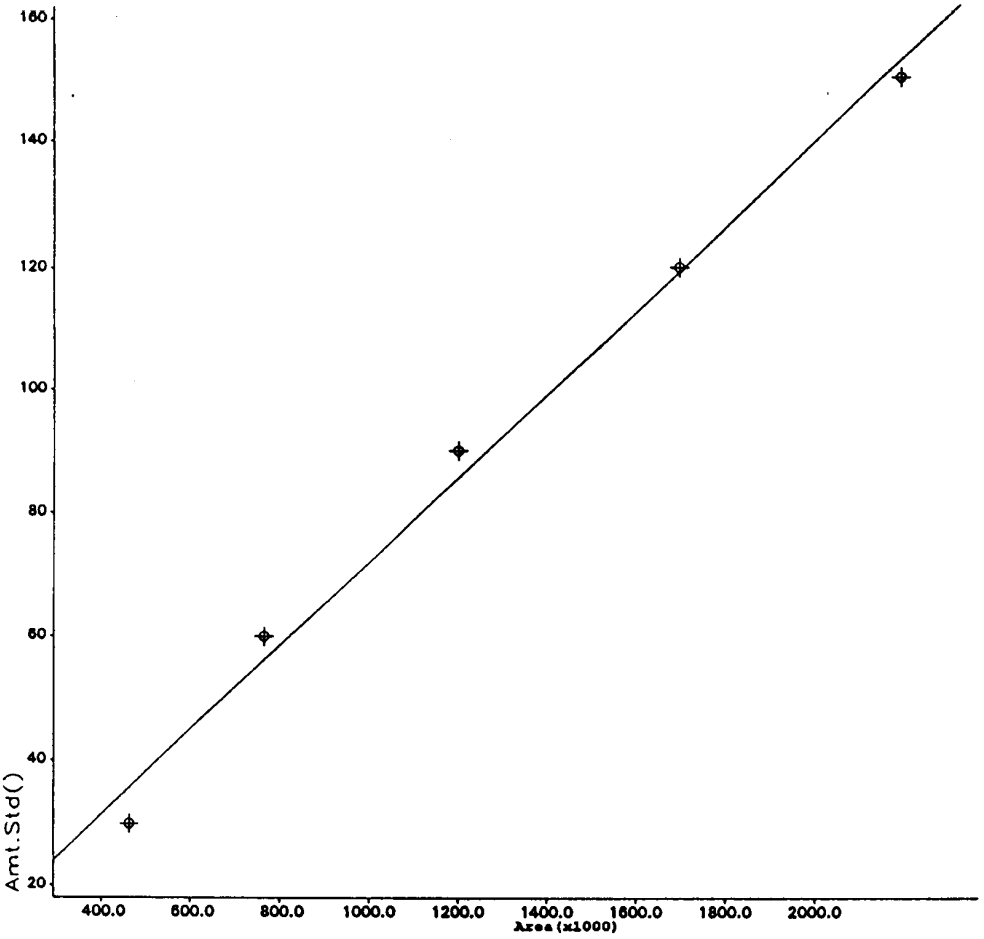
Regression Analysis:
Average Area = 1.1512E+006
Standard Deviation = +-8.9339E+003 (+- 0.78%)
Confidence Region = +-1.0542E+004

12 Result list of a multi-level-calibration
(here only shown for the first two peaks)

```
Multi Calibration Table        Date: 14.03.1996    Time:  9:07

Calculation Mode: External Standard

Standard Sample Table

Nr.  Report-File-Name
 1        qu4-0011
 2        qu4-0021
 3        qu4-0031
 4        qu4-0041
 5        qu4-0051


Peak Table
Peak Name: Toluol                          Time Window:   1.99
 Std.Amt           Area
==========================================================================
1.0000E+001         319709
                      319709
2.0000E+001         548247
                      548247
3.0000E+001         845240
                      845240
4.0000E+001        1180311
                     1180311
5.0000E+001        1528649
                     1528649
--------------------------------------------------------------------------
Regression Analysis
K0 = 1.1818E+000                    Coeff. of Correlation  = 0.996890
K1 = 3.2584E-005                    Std. Error of Estimat. = 1.245968
K2 = 0.0000E+000
K3 = 0.0000E+000
--------------------------------------------------------------------------

Peak Name: Ethylbenzol                          Time Window:   2.26
 Std.Amt           Area
==========================================================================
3.0000E+001         464142
                      464142
6.0000E+001         766108
                      766108
9.0000E+001        1199684
                     1199684
1.2000E+002        1696792
                     1696792
1.5000E+002        2193673
                     2193673
--------------------------------------------------------------------------
Regression Analysis
K0 = 4.3413E+000                    Coeff. of Correlation  = 0.995767
K1 = 6.7764E-005                    Std. Error of Estimat. = 4.360044
K2 = 0.0000E+000
K3 = 0.0000E+000
--------------------------------------------------------------------------
```

13 Graphical presentation of the 1st order calibration function
(here only shown for the second peak)

MULTILEVEL CALIBRATION Date: 14.03.1996 Time: 9:12
Peak Name : Ethylbenzol
Time Window : 2.26



Coefficients of Least Square Fit to a LINEAR equation
K0 : 4.3413E+000
K1 : 6.7764E-005

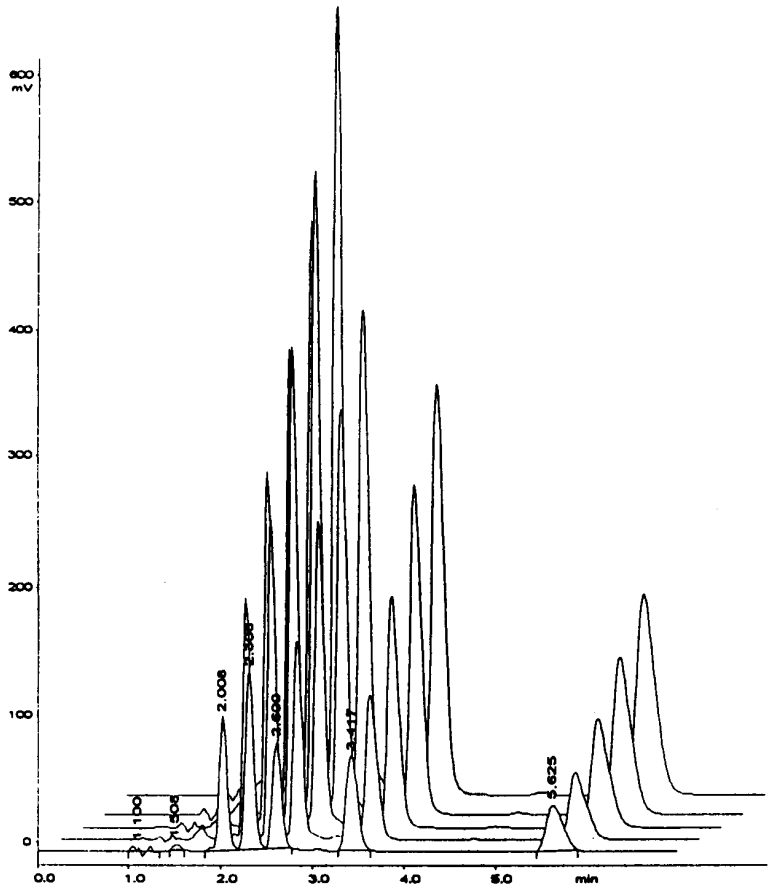
Coefficient of Determination : 0.991551
Correlation Coefficient : 0.995767

6.4 Print-outs of multiple chromatograms

14 3-Dimensional presentation of several chromatograms (Print template .RPD)

Display Chromatograms Date: 15.03.1996

qu4-0011
qu4-0021
qu4-0031
qu4-0041
qu4-0051

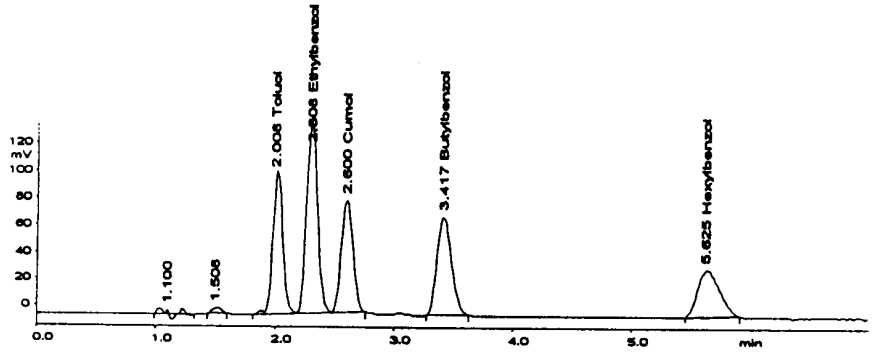


Page: 1 of: 1

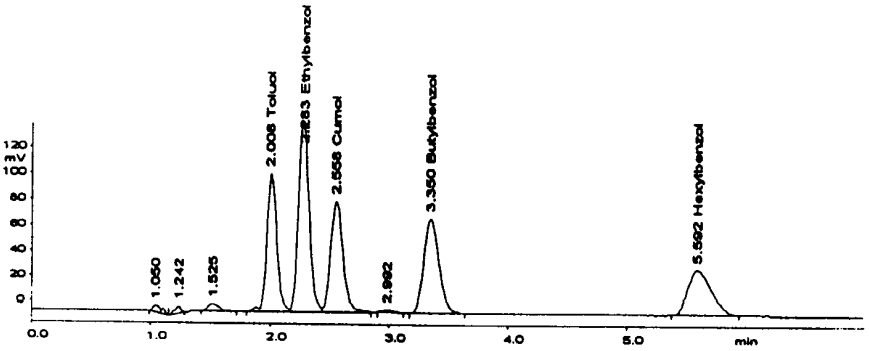
15 Presentation of 3 stacked chromatograms (Print template .RPC)

Compare Chromatograms Date: 17.04.1996

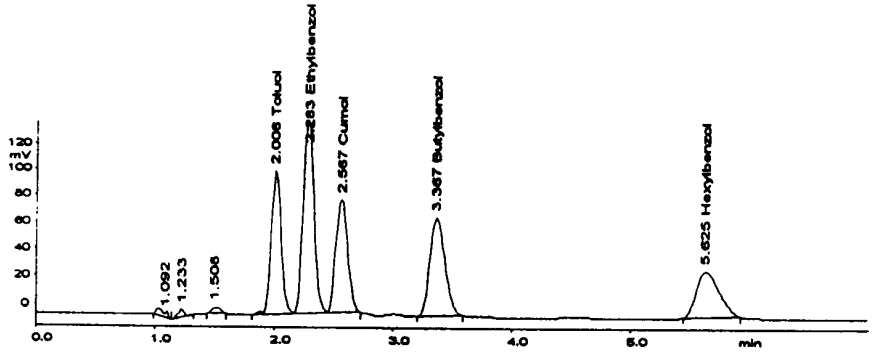
Chromatogram: qu4-0011 Sample Identifier: Testmix5



Chromatogram: qu4-0012 Sample Identifier: Testmix5



Chromatogram: qu4-0013 Sample Identifier: Testmix5



Seite: 1 von: 1

16 Print-out of chromatograms with multi-channel data acquisition (Print template .RPD)

Date: 18.04.1996 Time: 12:21

Author: H. Risler

Notes: Test CS40, AS, LC22, 2 Kanäle

Chromatogram: ne110032
Sample Identifier:
Injected on: 21.03.1996 Injected at: 12:14

Data Processing Parameters

Slice Width (msec): 500 Noise: 300
Threshold (mV): 500 Skim Ratio: 0

Parameter Files

Data Handling File: NEU1 Calculation-File:

Calculation Method : Percent

Peak-No	Ret.Time (min)	Pk.Start (min)	Pk.End (min)	Area	Height (mV)	Area%
1	0.708	0.617	0.750	3196	1.07	0.185
2	1.083	1.017	1.125	15972	7.53	0.923
3	1.283	1.125	1.408	97727	18.19	5.647
4	1.533	1.408	1.600	14383	3.70	0.831
5	1.758	1.658	1.942	934081	407.97	53.970
6	2.000	1.942	2.075	3778	1.98	0.218
7	2.217	2.158	2.292	5946	2.83	0.344
8	2.617	2.492	2.883	655651	231.57	37.883
				1730734	674.84	100.000

Chromatogram: ne120032
Sample Identifier:
Injected on: 21.03.1996 Injected at: 12:14

Data Processing Parameters

Slice Width (msec): 500 Noise: 400
Threshold (mV): 500 Skim Ratio: 3

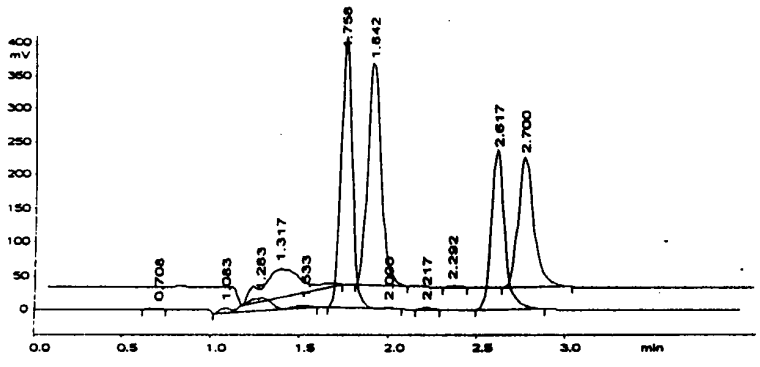
Parameter Files

Data Handling File: NEU2 Calculation-File:

Calculation Method : Percent

Peak-No	Ret.Time (min)	Pk.Start (min)	Pk.End (min)	Area	Height (mV)	Area%
1	1.317	1.100	1.667	353943	42.36	18.594
2	1.842	1.733	2.033	908323	329.54	47.718
3	2.292	2.233	2.375	5958	3.49	0.313
4	2.700	2.567	2.967	635286	190.13	33.374
				1903510	564.52	100.000

Page: 1 of: 2



6.5 Print-outs processed in chapter 5 (Chromatography with ChromStar)

17 Result list without grouping and summation

Chromatogram: -IBA0101 Date: 18.04.1996 Time: 8:05

Author:

Notes:

Sample Identifier: Ciba Basel R
Injected on: 05/03/87 Injected at: 00:00

Data Processing Parameters

Slice Width (msec): 500 Noise: 800
Threshold (mV): 500 Skim Ratio: 3

Parameter Files

Data Handling File: CIBA- Calculation-File:

Calculation Method : Percent

Peak-No	Ret.Time (min)	Pk.Start (min)	Pk.End (min)	Area	Height (mV)	Areat
1	0.308	0.217	0.350	90829	44.16	0.205
2	0.467	0.350	1.033	1709127	411.78	3.850
3	0.958	0.808	1.033	380028	89.21	0.856
4	1.200	1.033	1.692	5510110	964.33	12.411
5	1.467	1.358	1.692	532243	109.76	1.199
6	1.817	1.692	1.958	125395	25.15	0.282
7	2.250	2.158	2.333	24880	8.40	0.056
8	2.700	2.567	2.758	59401	15.82	0.134
9	2.900	2.758	3.167	286461	52.21	0.645
10	4.700	4.467	5.000	2154290	403.69	4.852
11	5.183	5.000	5.325	184155	29.62	0.415
12	5.500	5.325	5.775	218645	29.38	0.492
13	6.650	6.508	6.808	65799	13.23	0.148
14	8.042	7.875	8.217	81145	14.31	0.183
15	11.683	11.458	11.917	416052	71.26	0.937
16	12.942	12.758	13.333	794228	110.37	1.789
17	16.942	16.667	17.225	1022925	148.95	2.304
18	17.658	17.450	17.842	239351	42.06	0.539
19	18.708	18.575	18.892	84508	16.88	0.190
20	19.767	19.367	20.050	9005606	936.60	20.285
21	20.225	20.050	20.408	102626	18.52	0.231
22	21.008	20.808	21.225	222143	36.08	0.500
23	22.392	22.208	22.492	88437	15.54	0.199
24	22.658	22.492	22.833	167900	28.63	0.378
25	24.650	24.392	24.925	2811236	511.44	6.332
26	25.433	25.308	25.567	46131	10.70	0.104
27	25.925	25.808	26.042	30726	7.67	0.069
28	26.417	26.242	26.575	96811	19.33	0.218
29	27.975	27.783	28.192	360845	63.01	0.813
30	28.608	28.500	28.750	47878	11.66	0.108
31	29.483	29.300	29.817	410672	61.97	0.925
32	30.883	30.692	31.042	532241	115.08	1.199
33	31.225	31.042	31.592	846682	149.00	1.907
34	31.758	31.592	31.867	45735	11.54	0.103
35	32.342	32.075	32.683	5857524	922.32	13.194
36	32.883	32.683	32.992	94283	25.43	0.212
37	33.167	33.100	33.342	25748	12.62	0.058
38	34.033	33.667	34.258	2001137	319.59	4.507
39	34.425	34.325	34.508	42755	10.79	0.096
40	34.625	34.508	34.733	84070	17.28	0.189
41	34.867	34.733	35.100	168361	30.87	0.379
42	35.358	35.100	35.575	340144	49.60	0.766
43	37.375	37.167	37.483	650035	122.26	1.464
44	37.542	37.483	37.758	239331	64.91	0.539
45	38.125	37.892	38.583	3738435	820.18	8.421
46	39.033	38.792	39.317	951909	132.26	2.144
47	39.558	39.467	39.717	45609	11.32	0.103
48	42.533	42.267	42.750	210718	27.06	0.475
49	43.892	43.525	44.300	1150778	120.38	2.592
				44396080	7284.22	100.000

18 Result list with grouping and summation

Chromatogram: -IBA0101 Date: 18.04.1996 Time: 8:06

Author:

Notes:

Sample Identifier: Ciba Basel R
Injected on: 05/03/87 Injected at: 00:00
Data Processing Parameters
Slice Width (msec): 500 Noise: 800
Threshold (mV): 500 Skim Ratio: 3
Parameter Files
Data Handling File: CIBA- Calculation-File: CIBA3

Calculation Method : Percent

Peak-No	Win No.	Ret.Time (min)	Area	Resp.-Fact.	Areat	Name
1		0.308	90829	1.000	0.205	
2		0.467	1709127	1.000	3.850	
3		0.958	380028	1.000	0.856	
4		1.200	5510110	1.000	12.411	
5		1.467	532243	1.000	1.199	
6		1.817	125395	1.000	0.282	
7		2.250	24880	1.000	0.056	
8		2.700	59401	1.000	0.134	
9		2.900	286461	1.000	0.645	
10	1	5.128	2557089	1.000	5.760	Gruppe1
13		6.650	65799	1.000	0.148	
14		8.042	81145	1.000	0.183	
15	2	12.313	1210280	1.000	2.726	Gruppe2
17		16.942	1022925	1.000	2.304	
18		17.658	239351	1.000	0.539	
19		18.708	84508	1.000	0.190	
20	3	19.767	18601564	1.000	41.899	Summe1
21		20.225	102626	1.000	0.231	
22		21.008	222143	1.000	0.500	
23		22.392	88437	1.000	0.199	
24		22.658	167900	1.000	0.378	
25	4	24.650	4812373	1.000	10.840	Summe2
26		25.433	46131	1.000	0.104	
27		25.925	30726	1.000	0.069	
28		26.417	96811	1.000	0.218	
29		27.975	360845	1.000	0.813	
30		28.608	47878	1.000	0.108	
31		29.483	410672	1.000	0.925	
32		30.883	532241	1.000	1.199	
33		31.225	846682	1.000	1.907	
34		31.758	45735	1.000	0.103	
36		32.883	94283	1.000	0.212	
37		33.167	25748	1.000	0.058	
39		34.425	42755	1.000	0.096	
40		34.625	84070	1.000	0.189	
41		34.867	168361	1.000	0.379	
42		35.358	340144	1.000	0.766	
43		37.375	650035	1.000	1.464	
44		37.542	239331	1.000	0.539	
46		39.033	951909	1.000	2.144	
47		39.558	45609	1.000	0.103	
48		42.533	210715	1.000	0.475	
49		43.892	1150778	1.000	2.592	
			44396080		100.000	

19 One-Point-Calibration in a current run

Chromatogram: QUAN0021 Date: 18.04.1996 Time: 8:58

Author: H. Risler

Notes:

Sample Identifier:

Injected on: 18.04.1996 Injected at: 8:58

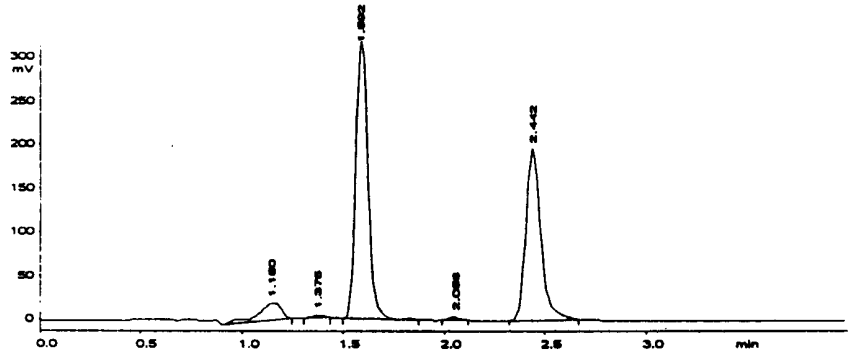
Data Processing Parameters

Slice Width (msec): 500 Noise: 500

Threshold (mV): 500 Skim Ratio: 3

Parameter Files

Data Handling File: QUAN Calculation-File: QUAN



Calculation Method : Calibration

Peak-No	Win No.	Ret.Time (min)	Area	Resp.-Fact.	Std.Amt. mg/100ml	Name
3	1	1.592	694182	7.2027E-05	50.000	Cumol
5	2	2.442	531493	2.8222E-04	150.000	Hexylbenzol
			1225675			

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20 Quantitative evaluation after a current run

Chromatogram: QUAN0031 Date: 18.04.1996 Time: 9:04

Author: H. Risler

Notes:

Sample Identifier:

Injected on: 18.04.1996 Injected at: 9:04

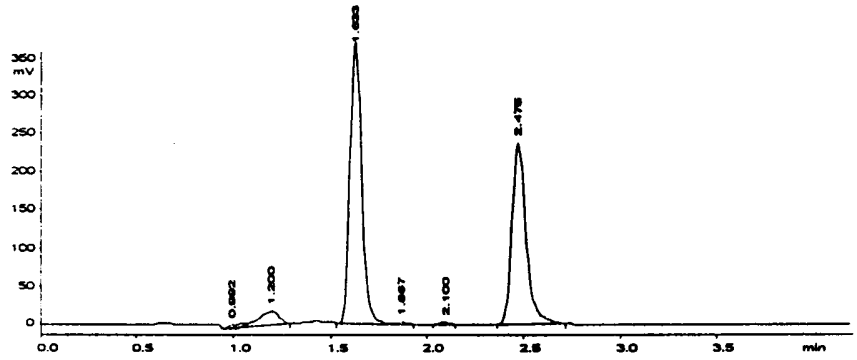
Data Processing Parameters

Slice Width (msec): 500 Noise: 500

Threshold (mV): 500 Skim Ratio: 3

Parameter Files

Data Handling File: QUAN Calculation-File: QUAN



Calculation Method : External Standard

Calibration Method : One Point

Peak-No	Win No.	Ret.Time (min)	Area	Resp.-Fact.	Absolute mg/100ml	Weight (%)	Name
3	1	1.633	808698	7.2027E-05	58.248	0.000	Cumol
6	2	2.475	633015	2.8222E-04	178.652	0.000	Hexylbenzol
			1441714		236.900		

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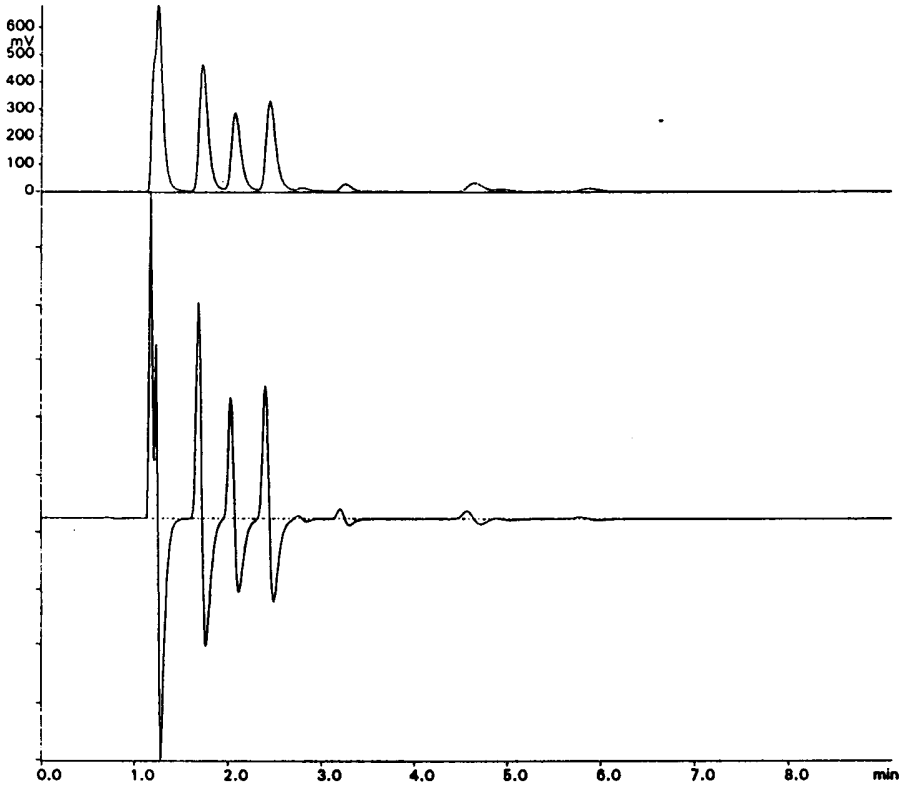
6.6 Print-outs of chromatograms after File-Transformation

21 1st derivative of a chromatogram

Current user : H. Risler Date: 17.04.1996 Time: 11:45

FILE TRANSFORMATION

Operation : 1.Derivative
Chromatogram : AROM0001

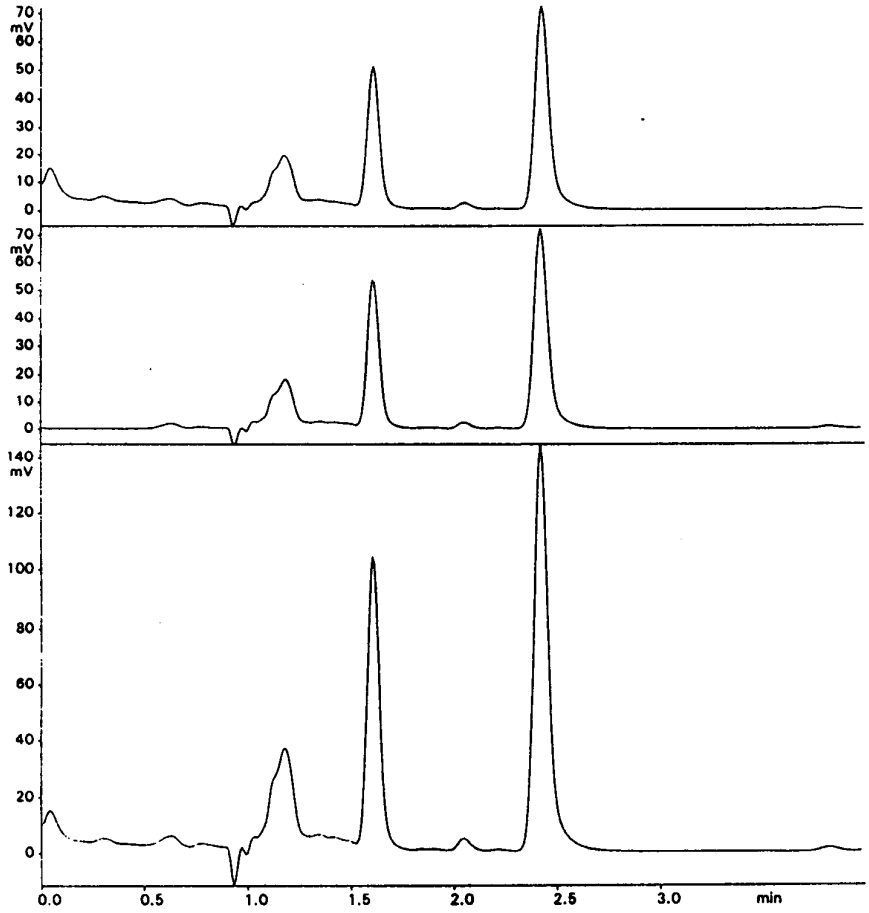


22 Addition of two chromatograms

Current user : H. Risler **Date:** 17.04.1996 **Time:** 11:52

FILE ARITHMETICS

Operation : **Addition**
Reference Chromatogram : **N2000011**
Work Chromatogram : **N2000012**



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